Biodiversity and ecosystem functioning in stressed environments:

primary producers and consumers at the basis of marine food webs

GHENT UNIVERSITY

FACULTY OF SCIENCES

Marine Biology Research Group

Campus Sterre - S8

Krijgslaan 281

B-9000 Gent

Belgium

FACULTY OF BIOSCIENCE ENGINEERING

Laboratory for Environmental Toxicology and Aquatic Ecology
Environmental Toxicology Unit - GhEnToxLab

Coupure Links 653

B-9000 Gent

Belgium

Academic year 2016-2017

Publically defended on December 19th, 2016

Mensens C (2016) Biodiversity and ecosystem functioning in stressed environments: primary producers and consumers at the basis of marine food webs. Ghent Unversity (UGent).

For citation to published chapters in this thesis, please refer to the original publications.

Biodiversity and ecosystem functioning in stressed environments: primary producers and consumers at the basis of marine food webs

By Christoph Mensens

Promotor: Prof. Dr. Marleen De Troch

Co-promotors: Prof. Dr. Colin Janssen

Prof. Dr. Frederik De Laender

Prof. Dr. Jean-Marc Guarini

Academic year 2016-17

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor in Science:

Marine Sciences

This work was supported by a Ph.D. grant from the Doctoral Programme on Marine Ecosystem Health and Conservation (MARES) and by the UGent Special Research Fund (BOF).

Members of the examination committee and reading committee *

Prof. Dr. Marleen De Troch, Promotor
Ghent University, Ghent, Belgium

Prof. Dr. Colin Janssen, Co-promotor
Ghent University, Ghent, Belgium

Prof. Dr. Frederik De Laender, Co-promotor
Namur University, Namur, Belgium

Prof. Dr. Jean-Marc Guarini, Co-promotor
Université Pierre et Marie Curie, Paris, France

Dr. Francesco Pomati *
Eawag, Dübendorf, Switzerland

Prof. Dr. Tom Moens *
Ghent University, Ghent, Belgium

Prof. Dr. Koenraad Muylaert *
Catholic University of Leuven, Campus Kortrijk, Belgium

Prof. Dr. Koen Sabbe *
Ghent University, Ghent, Belgium

Prof. Dr. Nicolas Loeuille*
Université Pierre et Marie Curie, Paris, France

Prof. Dr. Ann Vanreusel *
Ghent University, Ghent, Belgium

Acknowledgements

The time has finally arrived to give my deepest gratitude and thanks to all the people that have made this PhD thesis possible.

Special mention goes to my promotor, Marleen De Troch. I would like to express my deepest gratitude for her continuous stimulating input, her availability and guidance, and all the time she has dedicated to correcting and improving my texts. I am grateful for the many constructive discussions we had over the past years, which shaped the conceptual framework of this thesis. Your working enthusiasm was contagious and motivational for me and, to stay in a BEF context, was the main mechanism that kept me functioning during the stressful and tough times of this thesis. I would like to say a big thank you to my co-promotor Frederik De Laender. I cannot say how much I appreciate your dedicated commitment towards this project from the beginning till the very end. Your extensive knowledge in BEF research was an essential contribution to the quality of this work. Thank you for repeatedly helping me and never losing patience when I was struggling with the modelling, and always guiding me in the right direction. I am very thankful to Colin Janssen for his continuous support, advice and encouragements over the last four years. I would like to thank Jean-Marc Guarini for his insightful comments and advising me on my way through the French academic system. I would also like to thank the members of my reading committee, Francesco Pomati, Koenraad Muylaert, Tom Moens, Koen Sabbe, Nicolas Loeuille and Ann Vanreusel, for the time and energy they have invested for improving the final manuscript of the thesis with their insightful comments and suggestions.

The members of the Marbiol group have contributed immensely to my personal and professional time at UGent. The group has been a source of friendships as well as good advice and collaboration. First of all, I would like to thank Magda Vincx and Ann Vanreusel for running this lab so inspiringly, and for providing the support and facilities which made this thesis possible. Among the many people who supported me over the last years, I would like to thank Dirk Van Gansbeke for all the help in analyzing endless diatom and copepod fatty acid samples. A big thank you goes to Thibaud Mascart, not only for being an incredible friend, but also for his help, professional and administrative advice without which I would have been lost during my first year in Belgium. I would like to thank Renata Mamede da Silva Alves for her help with my R codes and her competent advice in statistics. Eva Werbrouck for teaching me how to identify copepods and letting me use her copepod samples for an experiment, and for having me as an office mate for over four years. Carl Van Colen, for helping with sampling in Paulina, and for managing the lab's seawater supply, ensuring that there was always water available to keep the microcosms and diatom & copepod stock cultures growing. Tom Moens and Niels Viaene for helping me with copepod sampling and

extraction, respectively. Annelien Rigaux for helping me with my lab work, Bart Beuselinck for the organic carbon and nitrogen analyses, and Jeroen de Caesemaeker and Guy de Smet for keeping my computer and software up and running. Last but not least, my great appreciation goes to Annick Van Kenhove and Isolde De Grem, not only for the administrative support, but also for ensuring the constant and timely supply of lab material. Annick additionally guided me through the healthcare system after I broke my arm and I am more than grateful for that.

I am very thankful to the members of the Laboratory of Environmental Toxicology and Aquatic Ecology: Colin Janssen, for always encouraging and supporting me. Nancy De Saeyer, without whose extensive advice and practical help with the chemical work, the experiments in this thesis would not have been possible. Jan Baert, for sharing his R codes with me and thereby enabling me to realize the fourth chapter of this thesis, and David Deruytter for letting me use his stock solutions and lab material.

I would like to thank the members of the Protistology and Aquatic Ecology research group, who substantially contributed to the progression of this thesis. I am very appreciative to Koen Sabbe for our detailed and stimulating discussions, which contributed a lot towards refining the chapters of this thesis. I would like to thank Olga Chepurnova for the conscientious maintenance of the culture collection and for the valuable and continuous support in growing diatom cultures. I would like to thank Tine Verstraete and Ilse Daveloose for all the support in the culture lab and chemical lab. I am grateful to Pieter Vanormelingen and Bart Vanelslander for helping me to identify diatom species, start my diatom cultures, and finally all the advice they provided during my first months in the lab.

This work has been supported by a Ph.D. grant from the Joint Doctoral Programme on Marine Ecosystem Health & Conservation (MARES), funded through Erasmus Mundus (from 1st January 2013 to 31st December 2015). From 1st January to 30th November 2016, this work was funded by a finalizing Ph.D. grant of the UGent Special Research Fund (Bijzonder Onderzoeksfonds).

I owe a great debt of gratitude to the MARES and EMBC family. First and foremost, I would like to thank Magda Vincx and Tim Deprez for establishing the Erasmus Mundus master and PhD programmes and making them such a success. Their care and dedication towards their students always went beyond their role as organizers and coordinators. This thesis is only a tiny fraction of the large network of cooperation, research contacts and friendships which they have initiated, and which will live on for a long time in the future. A big thank you goes to Pieter Blondeel, not only for being a great company during the MARES events, but also for his and Veerle Rogge's meticulous and dedicated efforts to keep the MARES programme up and running.

My time in Ghent was made enjoyable in large part due to the many friends and groups who became a part of my life. You have contributed immensely to my luck and happiness here, and I am grateful for all the long discussions, travels, weekend trips, lunch break discussions, days in the park, nights out, discussions, and many more unforgettable experiences.

I am immensely grateful to my loving girlfriend Danae, whose unconditional love and support have formed the backbone and origin of my happiness. Although she had to bear the main burden of my work-related mood shifts, night sessions and escapist ideas, she never lost patience with me, spiced every single day with neverending care and love, and has made our life together the most fulfilling experience I can think of. Σ'αγαπώ, είσαι το αστέρι της ζωής μου!

This last word of acknowledgment I have saved of course for my parents, who have raised me with so much love and devotion. You are the best example of what is really important in life. I owe you an infinite debt of gratitude for the endless moral support during the good and hard times of the last nearly thirty years. Ich kann nicht ausdrücken, wie sehr ich euch liebe und alles, was ich bin, verdanke ich euch. Je ne peux exprimer à quel point je vous aime et tout ce que je suis, je vous le dois.

Table of contents

Summary

Biodiversity is increasingly altered by human activities and there is a growing concern to what extent biodiversity loss will affect ecological processes that are essential to ecosystem functioning and ultimately to human well-being. These concerns have made the relationship between **biodiversity and ecosystem functioning** (diversity-functioning hereafter) a core research area in ecology, and two decades of intensive research have provided compelling evidence for a link between biodiversity and ecosystem functioning.

Anthropogenic stressors such as an increasing number of chemical contaminants are the driving force behind the ongoing biodiversity loss. Whilst diversity-functioning research is often framed within the context of anthropogenic change, anthropogenic stressors are however rarely included into the design of diversity-functioning studies. Similarly, the majority of diversity-functioning experiments have focussed on single trophic levels, whereas the impact of anthropogenic stressors across trophic levels has remained underrepresented in diversity-functioning research.

This PhD used marine primary producers (**benthic diatoms**) and consumers (**harpacticoid copepods**) and two chemical stressors (atrazine and copper) to explore four central questions which are still largely debated or unresolved in current diversity-functioning research: (i) Does the classic design in diversity-functioning research, which consists of random species loss, predict the diversity gradients and diversity-functioning relations induced by anthropogenic stress? (ii) Can biodiversity loss under stress, and its impact on ecosystem functioning, be predicted from the species' traits? (iii) Is the biodiversity effect on functioning in stressed communities determined by the dominant species (dominance effect) or a better performance of species in community (complementarity effect), and which are the mechanisms driving both effects? (iv) Are stress effects on the energy content of primary producers (diet quality) transmitted to the consumer level? The main findings regarding these questions are presented in Chapters 2 to 5, whilst Chapter 6 proposes an integrative synthesis of the three key effects through which anthropogenic stress drives the diversity-functioning relation.

The majority of diversity-functioning experiments to date used broad gradients of species richness, and tested the effect of biodiversity loss on ecosystem functioning by removing species in a random way. It remains unsure however whether this classic protocol is representative of the biodiversity loss and diversity-functioning relations as induced by anthropogenic stress. The first objective of this work was therefore to contrast the biodiversity gradients and diversity-functioning relations in a benthic diatom community as obtained with the classic random design with those induced by atrazine (**Chapter 2**). Biodiversity was quantified as species richness and evenness, and functioning as the diatom's contribution to primary production, biomass

production, energy content and sediment stabilization. Biodiversity gradients induced by atrazine exposure were narrower than predicted by the classic random approach, since the herbicide altered evenness but did not cause any species loss. Diversity- functioning relations for sediment stabilization and energy content were steeper in stressed than in randomly assembled communities. This disproportionately large decrease of functioning with stressor-induced biodiversity loss was related to selective atrazine effects on the species contributing most to energy content and sediment stabilization (*Nitzschia* sp.), which was also most sensitive to stress. The common approach in diversity-functioning research thus overestimated biodiversity loss induced by chemical stressors, but underestimated the associated loss of function due to selective stress effects targeting the species contributing most to functioning.

Given the plethora of anthropogenic stressors, it is logistically impossible to predict selective stress effects by experimentally testing the tolerance of species to each of these stressors. **Chapter 3** therefore tested if response traits could respectively predict changes in the abundance (numerical response) and in the effect traits (functional response; potential contribution to primary production, sediment stabilization and energy content) of 18 marine benthic diatom strains under copper and atrazine. Furthermore, it was tested whether the species' numerical stress response was related to their functional stress response, i.e. if tolerant species could maintain their functional contribution under stress. The numerical and functional response of diatoms to copper was predicted by the same set of intercorrelated morphological traits (cell size, surface to volume ratio, cell length), with large cells being more resistant to the metal. Under copper, the diatoms' numerical and functional response were positively related, meaning that copper-tolerant species were little affected in their contribution to functioning. Under atrazine, the capacity for mixotrophic growth predicted the numerical but not the functional stress response of diatoms, and the diatoms' numerical response to atrazine was not related to their functional response. Diatoms capable of growing on organic carbonic sources could thus maintain their cell numbers, but contributed little to functioning under herbicide stress. Overall, Chapter 3 indicated that if numerical and functional stress response are predicted by the same set of response traits, numerically tolerant species might be able to maintain ecosystem functioning under stress. When traits driving species abundances do not allow to maintain the contribution to functioning, stress might cause a disproportionate loss of functioning.

Dominance (dominance by species with high or low functional contribution) and complementarity (species contribute more to functioning in community than in monoculture) are the two key effects driving the biodiversity effect on ecosystem functioning, but there is little evidence on the effects of anthropogenic stress on dominance and complementarity. Complementarity effects arise from ecological mechanisms such as niche partitioning or

facilitative interactions between species. Direct functional and ecophysiological tests of these mechanisms are however scarce and there is no evidence attributing changes of complementarity under stress to changing facilitative mechanisms. **Chapter 4** first examined whether the biodiversity effect on diatom biomass production under atrazine and copper stress was driven by dominance or complementarity effects. Next, it was tested if the release of extracellular polymers as a facilitative mechanism could explain changes in complementarity under stress. Last, it was analysed if complementarity was two-way or one-way, i.e. if complementarity was equally distributed among species, or favoured species depending on their properties. The biodiversity effect on diatom biomass production depended on two-way complementarity, which increased under stress. Under atrazine and copper stress, diatom communities increased their production of extracellular polymers which, in part, predicted increases in complementarity. Diatom species benefited from complementarity depending on their properties, with complementarity under atrazine and copper respectively favouring the growth of mixotrophic (*C. closterium, Nitzschia sp., N. acicularis*) and copper-sensitive species (*A. lineolata, N. digitoradiata, Gyrosigma* sp.). These copper-sensitive species were however characterised by a low biomass production, causing a negative one-way complementarity, which largely offset positive two-way complementarity effects and limited the biodiversity effect on diatom biomass production under metal stress. Chapter 4 provided first ecophysiological evidence for facilitation as driver of complementarity under stress, by identifying extracellular polymers as a 'sleeping' facilitative mechanism, which when stimulated under stress predicted the diversity effect on biomass production. This facilitative mechanism was however not necessarily beneficial for ecosystem functioning when it caused a negative one-way complementarity which benefited species with a low functional contribution.

There is little evidence whether stressors drive functioning through selective effects (changes in community structure) or context-dependent effects (changes in the species' functional contribution), and if both types of stress knock on across trophic levels. Moreover, trophic approaches to date have essentially focussed on diet quantity, i.e. primary producer biomass, whilst diet quality (producer energy content) has not been considered when examining food web functioning under stress. **Chapter 5** measured if atrazine and copper affected functioning in a diatom community (diet quantity, sediment stabilization, diet quality) through selective stress effects (by selectively targeting the species contributing most to functioning) or context-dependent effects (by changing the species' functional contribution). Concomitant knock-on effects of selective and context-dependent stress effects on the next trophic level were quantified by testing the response of the diatoms' main grazer (the harpacticoid copepod *M. littorale*) to changes in diet quality. Diatom diet quantity was reduced by copper stress but not by low atrazine levels due to the presence of an atrazine-tolerant, mixotrophic species (*C. closterium*). The

diatoms' contribution to sediment stabilization was stimulated by context-dependent effects of both stressors. At low atrazine levels, selective changes in community structure involving dominance by the atrazine-tolerant but energy-poor species *C. closterium*, reduced diet quality by more than half. Context-dependent stress effects only reduced diet quality at high atrazine and copper levels. Furthermore, selective and context-dependent stress effects on diet quality affected the energy transfer to the next trophic level, with *M. littorale* losing half of its energy content when feeding on diatoms grown under atrazine and high copper stress. Chapter 5 identified selective stress effects, causing shifts in community structure towards dominance by species with low functional contribution, as a more potent threat for ecosystem functioning than any direct stress effects on the species' functional contribution. The energy content of copepods depended on that of their diatom diet, highlighting the relevance of diet quality as a key driver of energy transfer at the primary producer-consumer interface.

Chapter 6 integrates the three main effects influencing ecosystem functioning under stress: the functional impact of selective stress, targeting the functionally most important species, increases with differences in the species' functional contribution, and can be predicted from the species' response and effect traits. Physiological stress, by directly reducing the species' functional contribution, drives ecosystem functioning through context-dependent effects at high stress levels. Increases in complementarity, driven by the activation of facilitative mechanisms under stress, can enable primary producers to maintain their functional contribution and limit losses of ecosystem functioning. By highlighting the three key effects driving the diversity-functioning relation under stress, this work proposes an conclusive framework for a better integration of anthropogenic stressors within diversity-functioning research.

Samenvatting

Biodiversiteit wordt in toenemende mate veranderd door menselijke activiteiten, en er is een groeiende bezorgdheid in hoeverre het verlies aan biodiversiteit invloed zal hebben op ecologische processen die essentieel zijn voor het functioneren van ecosystemen en uiteindelijk voor het menselijk welzijn. Deze bekommering zorgde ervoor dat de relatie tussen **biodiversiteit en het functioneren van ecosystemen** (diversiteit - ecosysteem functionering) een kernonderzoeksgebied in de ecologie werd, en twee decennia van intensief onderzoek hebben overtuigend bewijs geleverd voor een link tussen biodiversiteit en het functioneren van ecosystemen.

Antropogene stressoren zoals een toenemend aantal chemische verontreinigingen zijn de drijvende kracht achter het voortdurende verlies aan biodiversiteit. Terwijl onderzoek naar diversiteit – ecosysteem functionering vaak gekaderd wordt in de context van antropogene veranderingen, worden de antropogene stressoren waardoor het voortdurende verlies aan biodiversiteit echter zelden opgenomen in het ontwerp van diversiteit-functioneringsstudies. De meeste diversiteit-functioneringsexperimenten richten zich op één trofische niveau, terwijl de invloed van antropogene druk over trofische niveaus heen ondervertegenwoordigd is in diversiteit-functioneringsonderzoek.

Dit doctoraat gebruikt mariene primaire producenten (**benthische diatomeeën**) en grazers (**harpacticoide copepoden**) en twee chemische stressoren (atrazine en koper) om vier centrale vragen die nog steeds grotendeels worden betwist of onopgelost zijn in het huidige onderzoek te toetsen: (i) voorspelt het klassieke ontwerp in diversiteit-functioneringsonderzoek, dat bestaat uit een willekeurig soortenverlies, de diversiteitsgradiënten en diversiteit-functionering relaties veroorzaakt door antropogene stress? (ii) kan verlies van biodiversiteit onder stress en zijn impact ervan op het functioneren van ecosystemen, worden voorspeld op basis van kenmerken van de soort ? (iii) is het biodiversiteitseffect op het functioneren van gemeenschappen onder stress bepaald door de dominante soort (dominantie-effect) of een betere prestatie van soorten in de gemeenschap (complementariteitseffect), en welke zijn de mechanismen achter beide effecten? (iv) worden stresseffecten op de energie-inhoud van primaire producenten (dieetkwaliteit) doorgegeven naar het niveau van de consument? De belangrijkste bevindingen ten aanzien van deze vragen worden gepresenteerd in de hoofdstukken 2 tot 5, terwijl hoofdstuk 6 een integratieve synthese geeft van de drie belangrijkste effecten waardoor antropogene stress de diversiteit-functioneringsrelatie drijft.

Het merendeel van de diversiteit-functioneringsexperimenten gebruikte tot op heden een ruime gradiënt van de soortenrijkdom, en testte het effect van het verlies aan biodiversiteit op het

functioneren van ecosystemen door het verwijderen van soorten op een willekeurige manier. Het blijft echter onzeker of dit klassieke protocol representatief is voor het verlies aan biodiversiteit en de diversiteit-functioning relaties veroorzaakt door antropogene stress. De eerste doelstelling van dit onderzoek was dan ook om de biodiversiteitsgradiënten en diversiteit-functioneringsrelaties te contrasteren in een benthische diatomeeëngemeenschap zoals verkregen met de klassieke willekeurig ontwerp versus een gemeenschap geïnduceerd door atrazine (**Hoofdstuk 2**). Biodiversiteit werd gekwantificeerd als soortenrijkdom en 'evenness' (spreiding van de individuen over de soorten), en functionering in termen van de bijdrage van de diatomeeën aan de primaire productie, de productie van biomassa, energie-inhoud en sedimentstabilisatie. Biodiversiteitsgradiënten die veroorzaakt werden door atrazineblootstelling waren kleiner dan voorspeld door de klassieke willekeurige aanpak, omdat het herbicide de spreiding van de individuen over de soorten ('evenness') wijzigde maar geen verlies aan soorten veroorzaakte. Diversiteit-functioneringsrelaties voor sedimentstabilisatie en energie-inhoud waren steiler in gestresseerde dan in willekeurig samengestelde gemeenschappen. Deze onevenredig grote vermindering van functioneren met stress-geïnduceerd verlies van biodiversiteit was gelinkt aan selectieve atrazine effecten op de soort die het meest bijdroeg tot de energie-inhoud en sedimentstabilisatie (*Nitzschia* sp.). Dit was tevens de meest stressgevoelige soort. De algemene aanpak in diversiteit-functioneringsonderzoek overschat dus het verlies aan biodiversiteit veroorzaakt door chemische stressoren, maar onderschat het daarmee gepaard gaande verlies van functie als gevolg van selectieve stresseffecten gericht op de soorten die het meest bijdragen tot het functioneren.

Gezien de overvloed van antropogene stressoren is het logistiek onmogelijk om selectieve stresseffecten te voorspellen door de tolerantie van soorten experimenteel te testen voor elk van deze stressoren. **Hoofdstuk 3** testte daarom of de responseigenschappen en effecteigenschappen respectievelijk de abundantie (numerieke respons) en het functioneren (functionele respons; potentiële bijdrage aan de primaire productie, sedimentstabilisatie en energie-inhoud) kunnen voorspellen van 18 mariene benthische diatomeeënstammen tegenover koper en atrazine. Verder werd onderzocht of numerieke stressreactie van soorten gerelateerd was aan hun functionele bijdrage en hun functionele stressrespons. De numerieke en functionele respons van diatomeeën ten opzichte van koper werd voorspeld door dezelfde set gecorreleerde morfologische kenmerken (celgrootte, oppervlakte-volumeverhouding, cellengte), met grote cellen die beter bestand zijn tegen koper. In de koperbehandelingen was de numerieke stressrespons van de diatomeeën positief gerelateerd aan hun functionele bijdrage en functionele stressrespons, wat betekent dat de koper-tolerante soorten het meest bijgedroegen tot het functioneren en het best presteerden bij het handhaven van hun functie. Bij de atrazinebehandelingen voorspelde de mixotrofe groei de numerieke maar niet de functionele stressreactie van diatomeeën en de

numerieke respons van de diatomeeën ten opzichte van atrazine was niet gerelateerd met hun functionele bijdrage en functionele stressreactie. Diatomeeën die kunnen groeien op organische koolzuurbronnen kunnen dus celdensiteiten behouden, maar dragen weinig bij tot het functioneren onder herbicidestress. Over het algemeen, onderstreept Hoofdstuk 3 dat als numerieke en functionele reactie op stress worden voorspeld door dezelfde set van de respons- en effecteigenschappen, numeriek tolerante soorten mogelijks het ecosysteem functioneren kunnen behouden onder stress. Als respons- en effecteigenschappen niet overeenstemmen dan kan stress een onevenredig verlies van functioneren veroorzaken.

Dominantie (dominantie van soorten met een hoge of lage functionele bijdrage) en complementariteit (soorten die meer bijdragen aan het functioneren in de gemeenschap dan in monocultuur) zijn de twee belangrijkste effecten die het effect van biodiversiteit op ecosysteemfunctionering sturen maar er is weinig bewijs voor de effecten van antropogene druk op dominantie en complementariteit. Complementariteitseffecten vloeien voort uit de ecologische mechanismen zoals nichepartitionering of faciliterende interacties tussen soorten. Directe functionele en ecofysiologische testen van deze mechanismen zijn echter schaars en er is geen bewijs die wijzigingen in complementariteit onder stress kan toewijzen aan veranderende faciliterende mechanismen. **Hoofdstuk 4** onderzocht eerst of het biodiversiteitseffect op de productie van biomassa door diatomeeën onder atrazine- en koperstress werd gedreven door dominantie of complementariteitseffecten. Vervolgens werd onderzocht of de afgifte van extracellulaire polymeren als faciliterende mechanisme een verklaring kan zijn voor veranderingen in complementariteit onder stress. Tenslotte werd geanalyseerd of de complementariteit in twee richtingen of één richting voorkwam, met andere woorden of de complementariteit evenredig verdeeld werd tussen soorten, of of soorten bevoordeeld werden op basis van hun eigenschappen. Het biodiversiteitseffect op de productie van biomassa door diatomeeën was afhankelijk van twee-richtingen complementariteit, die toenam onder stress. Onder atrazine en koperstress verhoogden de diatomeeëngemeenschappen hun productie van extracellulaire polymeren wat deels de toename in complementariteit voorspelde. Diatomeeënsoorten profiteerden van complementariteit afhankelijk van hun eigenschappen, waarbij complementariteit bij behandeling met atrazine en koper de groei van respectievelijk mixotrofe (*C. closterium, Nitzschia* sp., *N. acicularis*) en kopergevoelige soorten (*A. lineolata, N. digitoradiata, Gyrosigma* sp.) promootte. Deze kopergevoelige soorten werden echter gekenmerkt door lage biomassa-opbrengsten, waardoor een negatieve éénrichtingscomplementariteit, die grotendeels positieve tweerichtingscomplementariteit effecten teniet deed en beperkte het biodiversiteitseffect op de productie van diatomeeënbiomassa bij stress door metalen. Hoofdstuk 4 leverde het eerste ecofysiologisch bewijs voor facilitatie als drijfveer van complementariteit onder stress, door het aanduiden van extracellulaire polymeren als een ‘slapend’ faciliterend

mechanisme, dat wanneer gestimuleerd onder stress het diversiteitseffect op de biomassaproductie voorspelde. Deze faciliterende mechanisme was echter niet per se gunstig voor het functioneren van het ecosysteem indien het een negatieve éénrichtingscomplementariteit veroorzaakte die soorten bevoordeligde met een lage functionele bijdrage.

Er is weinig bewijs dat stressoren functionering sturen door middel van selectieve effecten (veranderingen in de gemeenschapsstructuur) of context-afhankelijke effecten (veranderingen in functionele bijdrage van de soorten), en of beide types van stress een impact hebben over trofische niveaus heen. Bovendien hebben trofische benaderingen tot op heden zich voornamelijk gericht op dieethoeveelheid, dat wil zeggen de biomassa van de primaire producent, terwijl de kwaliteit van het dieet (energie-inhoud van de producent) niet beschouwd werd bij het onderzoek van het functioneren van een voedselweb onder stress. In **Hoofdstuk 5** werd onderzocht of atrazine en koper een effect hadden op het functioneren van een diatomeeëngemeenschap (dieethoeveelheid, sedimentstabilisatie, dieetkwaliteit) door middel van selectieve stresseffecten (door selectieve impact op de soorten die het meest bijdragen tot het functionering) of context-afhankelijke effecten (door het veranderen van de functionele bijdrage van de soorten). Gelijktijdig knock-on effecten van selectieve en context-afhankelijke stress effecten op de volgende trofische niveau werden gekwantificeerd door het testen van de respons van de belangrijkste grazers op de diatomeeën (de harpacticoide copepode *M. littorale*) ten opzichte van veranderingen in de dieetkwaliteit. De hoeveelheid van het diatomeeëndieet werd verlaagd door koperstress maar niet door lage atrazineniveaus door de aanwezigheid van een atrazine-tolerante, mixotrofe soort (*C. closterium*). De bijdrage van diatomeeën aan sedimentstabilisatie werd gestimuleerd door context-afhankelijke effecten van beide stressoren. Bij lage atrazineniveaus, verminderden selectieve veranderingen in de gemeenschapsstructuur met dominantie van de atrazine-tolerante maar energie-arme soort *C. closterium*, de dieetkwaliteit met meer dan de helft. Context-afhankelijke stresseffecten alleen beïnvloedden het functioneren van diatomeeën bij hoge atrazine- en koperniveaus. Selectieve en context-afhankelijke stresseffecten op dieetkwaliteit beïnvloeden de energietransfer naar het volgende trofische niveau, met *M. littorale* die de helft van zijn energie-inhoud verliest wanneer gevoed met diatomeeën opgegroeid onder atrazine- en hoge koperstress. Hoofdstuk 5 duidt selectieve stresseffecten aan, die verschuivingen in de gemeenschapsstructuur veroorzaken met een dominantie van soorten met een lage functionele bijdrage, als een meer potente bedreiging voor het functioneren van ecosystemen dan directe stresseffecten op de functionele bijdrage van de soort. De energie-inhoud van de copepoden hing af van die van hun diatomeeënvoedsel, dit onderlijnt de relevantie van de kwaliteit van het dieet als een belangrijke motor van de energie-overdracht op de primaire producent en consument interactie.

Hoofdstuk 6 integreert de drie hoofdeffecten die ecosysteemfunctionering beïnvloeden onder stress: de functionele impact van selectieve stress, gericht op de functioneel belangrijke soorten, verhoogt met de verschillen in de functionele bijdrage van de soort, en kan worden voorspeld op basis van de respons van de soort en de effectkenmerken. Fysiologische stress, door rechtstreeks verminderen van de functionele bijdrage van de soort, drijft ecosysteemfunctionering door middel van context-afhankelijke effecten bij hoge stressniveaus. Verhogingen van complementariteit, gedreven door de activering van faciliterende mechanismen onder stress, kunnen primaire producenten in staat stellen hun functionele bijdrage te behouden en beperken het verlies van het functioneren van ecosystemen. Door te wijzen op de drie belangrijkste effecten die de diversiteit-functioneringsrelatie onder stress sturen, stelt dit werk een integrale alomvattend kader voor om een betere integratie van antropogene stressoren binnen-diversiteit functionerende onderzoek te garanderen.

Résumé

La biodiversité est de plus en plus altérée par les activités humaines, et l'inquiétude envers les conséquences de la perte de biodiversité sur le fonctionnement des écosystèmes augmente, ce qui affecte, en fin de compte, le bien-être humain. Ces préoccupations ont fait de la relation entre **la biodiversité et le fonctionnement des écosystèmes** un domaine de recherche fondamental en écologie et deux décennies de recherches intensives ont fourni des preuves irréfutables d'un lien entre la biodiversité et le fonctionnement des écosystèmes.

Les **facteurs de stress anthropiques** comme par exemple le nombre croissant de contaminants chimiques sont une cause majeure de la perte de biodiversité. Si la recherche sur le fonctionnement des écosystèmes est souvent comprise dans le contexte de la perte de biodiversité causée par les activités humaines, les facteurs de stress anthropiques sont cependant rarement inclus dans les études sur la biodiversité et le fonctionnement des écosystèmes. De même, la majorité de ces études se sont concentrées sur des niveaux trophiques uniques, alors que l'impact des facteurs de stress anthropiques sur les niveaux trophiques est resté sous-représenté dans la recherche sur la biodiversité et le fonctionnement des écosystèmes.

Pour cette thèse de doctorat**,** ont été utilisés des producteurs primaires marins (**diatomées benthiques**) et des consommateurs (**copépodes harpacticoïdes**) et deux facteurs de stress chimique (atrazine et cuivre) pour explorer quatre questions centrales qui sont encore largement débattues ou non résolues dans la recherche actuelle: (i) le design classique de la recherche fonctionnant sur la diversité, qui consiste en une perte aléatoire d'espèces, laisse-t-il prévoir les gradients de diversité et les relations fonctionnant sur la diversité induites par le stress anthropique? (ii) La perte de biodiversité sous l'effet du stress et son impact sur le fonctionnement des écosystèmes peuvent-ils être prévus à partir des caractères de l'espèce? (iii) L'effet de la biodiversité sur le fonctionnement des communautés stressées est-il déterminé par l'espèce dominante (effet de dominance) ou par une meilleure performance des espèces en communauté (effet de complémentarité) et quels sont les mécanismes qui entraînent les deux effets? (iv) Les effets du stress sur le contenu énergétique des producteurs primaires (qualité de l'alimentation) sont-ils transmis au consommateur? Les principaux résultats concernant ces questions sont présentés dans les chapitres 2 à 5, tandis que le chapitre 6 propose une synthèse intégrative des trois effets-clés qui déterminent l'impact du stress anthropique sur la relation entre la biodiversité et le fonctionnement des écosystèmes.

La plupart des expériences fonctionnant sur la diversité ont jusqu'ici utilisé de larges gradients de richesse d'espèces et ont testé l'effet de la perte de biodiversité sur le fonctionnement de l'écosystème en supprimant des espèces de manière aléatoire. Il reste cependant incertain si ce

protocole classique est représentatif de la perte de biodiversité et des relations de fonctionnement de la diversité induites par le stress anthropique. Le premier objectif de ce travail était donc de comparer, dans une communauté de diatomées benthiques, les gradients de biodiversité et le fonctionnement des écosystèmes obtenus en utilisant le design aléatoire classique avec ceux induits par l'atrazine **(chapitre 2).** La biodiversité a été quantifiée en tant que richesse et régularité des espèces et le fonctionnement en tant que contribution des diatomées à la production primaire, à la production de biomasse, au contenu énergétique et à la stabilisation des sédiments. Les gradients de biodiversité induits par l'exposition à l'atrazine étaient plus étroits que prévus par le design aléatoire classique, étant donné que l'herbicide a altéré la régularité mais n'a causé aucune perte d'espèces. Les relations entre la diversité et le fonctionnement en tant que stabilisation des sédiments et contenu énergétique étaient plus accentuées que dans les communautés rassemblées de manière aléatoire. Cette diminution disproportionnée du fonctionnement avec la perte de biodiversité induite par le stress était liée aux effets sélectifs de l'atrazine sur l'espèce contribuant le plus au contenu énergétique et à la stabilisation des sédiments (*Nitzschia* sp.), qui était également le plus sensible au stress. Le design classique des études sur le fonctionnement de la diversité a donc surestimé la perte de biodiversité induite par le stress, mais a sous-estimé la perte de fonction due aux effets de stress sélectif ciblant l'espèce qui contribue le plus au fonctionnement.

Compte tenu de la pléthore de facteurs de stress anthropiques, il est logistiquement impossible de prévoir les effets du stress sélectif en testant expérimentalement la tolérance des espèces à chacun de ces facteurs de stress. Le **chapitre 3** a donc testé si les traits de réponse et les traits d'effet pourraient respectivement laisser prévoir l'abondance (réponse numérique) et le fonctionnement (réponse fonctionnelle, contribution potentielle à la production primaire, stabilisation des sédiments et teneur énergétique) de 18 souches de diatomées benthiques marines sous l'effet du cuivre et de l'atrazine. En outre, il a été testé si la réponse numérique au stress des espèces était liée à leur contribution fonctionnelle et à leur réponse fonctionnelle au stress, c'est-à-dire si l'atrazine et le cuivre ciblaient des espèces peu ou très importantes pour le fonctionnement des écosystèmes et si les espèces tolérantes pouvaient maintenir leur contribution fonctionnelle en situation de stress. La réponse numérique et fonctionnelle des diatomées au cuivre a été prédite par le même ensemble de traits morphologiques intercorrélés (volume et longueur des cellules, rapport surface / volume), les grandes cellules étant plus résistantes au métal. Dans le cas du cuivre, la réponse numérique des diatomées était positivement liée à leur contribution fonctionnelle et à leur réponse fonctionnelle, ce qui signifie que les espèces tolérantes au cuivre contribuaient le plus au fonctionnement et fonctionnaient le mieux sous l'effet du cuivre. Sous atrazine, la capacité de croissance mixotrophe laissait prévoir la réponse numérique, mais non fonctionnelle, des diatomées, et la réponse numérique des

diatomées à l'atrazine n'était ni liée à leur contribution fonctionnelle ni à leur réponse au stress fonctionnel. Les diatomées capables de croître sur des sources organiques carboniques ont ainsi pu maintenir leur abondance, mais elles ont peu contribué au fonctionnement sous stress herbicide. Dans l'ensemble, le chapitre 3 indique que si la réponse au stress numérique et fonctionnel est liée aux mêmes traits de réponse et d'effet, les espèces capables de maintenir leur abondance pourraient maintenir le fonctionnement de l'écosystème sous stress. Si les traits de réponse et d'effet ne correspondent pas, le stress pourrait entraîner une perte disproportionnée de fonctionnement.

La dominance (dominance par les espèces ayant une contribution fonctionnelle élevée ou faible) et la complémentarité (les espèces contribuent davantage au fonctionnement en communauté que dans la monoculture) sont les deux forces motrices principales de l'effet de la biodiversité sur le fonctionnement des écosystèmes. Par contre, les effets du stress anthropique sur la dominance et la complémentarité restent inconnus. Les effets de complémentarité découlent de mécanismes écologiques tels que le partage de niches ou les interactions facilitatrices entre espèces. Les tests fonctionnels et écophysiologiques directs de ces mécanismes sont toutefois rares et il n'existe aucune preuve reliant des changements de complémentarité en situation de stress à des interactions facilitatrices. Le **chapitre 4** a examiné en premier si l'effet de la biodiversité sur la production de biomasse de diatomées sous atrazine et cuivre était déterminé par des effets de dominance ou de complémentarité. Ensuite, il a été testé si la libération de polymères extracellulaires comme mécanisme de facilitation pourrait expliquer les changements de complémentarité sous stress. Pour finir, il a été analysé si la complémentarité était bidirectionnelle ou unidirectionnelle, c'est-à-dire si la complémentarité était répartie de manière égale entre les espèces ou favorisait les espèces selon leurs propriétés. L'effet de la biodiversité sur la production de biomasse des diatomées dépendait de la complémentarité bidirectionnelle, cette dernière ayant augmenté sous le stress. Les communautés de diatomées stressées par atrazine et cuivre ont augmenté leur production de polymères extracellulaires, causant, en partie, des augmentations de complémentarité. Les espèces de diatomées ont bénéficié de la complémentarité en fonction de leurs propriétés, la complémentarité sous atrazine et cuivre favorisant respectivement la croissance des espèces mixotrophes (*C. Closterium, Nitzschia* sp., *N. acicularis*) et sensibles au cuivre (*A. lineolata, N. digitoradiata, Gyrosigma* sp.). Ces espèces sensibles au cuivre étaient cependant caractérisées par une faible production de biomasse, entraînant une complémentarité négative à sens unique. Ceci a compensé la complémentarité bidirectionnelle positive et a limité l'effet de la biodiversité sur la production de biomasse de diatomées sous stress métallique. Le chapitre 4 a apporté les premières preuves écophysiologiques de la facilitation en tant que mécanisme provoquant la complémentarité sous stress. Les polymères extracellulaires ont donc été identifiés comme un mécanisme qui, stimulé

sous stress, laissait prévoir l'effet de diversité sur la production de biomasse. Ce mécanisme de facilitation n'était toutefois pas nécessairement bénéfique au fonctionnement, dans les cas où il a entrainé une complémentarité négative à sens unique qui a bénéficié aux espèces à faible contribution fonctionnelle.

Il y a peu de preuves permettant de déterminer si les facteurs de stress conduisent au fonctionnement par des effets sélectifs (changements dans la structure de la communauté) ou par des effets dépendant du contexte (changements dans la contribution fonctionnelle de l'espèce) et si les deux types de stress ont des effets à travers les niveaux trophiques. De plus, les études trophiques ont, à ce jour, essentiellement porté sur la quantité alimentaire, c'est-à-dire la biomasse des producteurs primaires, alors que la qualité alimentaire (contenu énergétique du producteur) n'a pas été prise en considération. Dans le **chapitre 5**, il a été examiné si l'atrazine et le cuivre affectaient le fonctionnement dans une communauté de diatomées (quantité alimentaire, stabilisation des sédiments, qualité alimentaire) par des effets de stress sélectif (en ciblant sélectivement les espèces qui contribuent le plus au fonctionnement) ou par des effets dépendant du contexte (en changeant la contribution fonctionnelle des espèces). Les effets du stress sélectif et du stress dépendant du contexte au niveau trophique suivant ont été examinés en testant la réponse du brouteur principal des diatomées (le copépode harpacticoïde *M. littorale*) aux changements de la qualité alimentaire. La quantité alimentaire des diatomées a été réduite par le stress du cuivre, mais pas par des concentrations faibles d'atrazine en raison de la présence d'une espèce mixotrophe tolérante à l'atrazine (*C. closterium*). La contribution des diatomées à la stabilisation des sédiments a été stimulée par les effets de stress dépendant du contexte. À de basses concentrations d'atrazine, des changements sélectifs dans la structure de communauté impliquant la dominance de l'espèce *C. closterium*, tolérante à l'atrazine, mais pauvre en qualité nutritionnelle, ont réduit la qualité alimentaire de plus de la moitié. Les effets de stress dépendant du contexte ont seulement réduit la qualité alimentaire à des niveaux élevés d'atrazine et de cuivre. En modifiant la qualité alimentaire, le stress sélectif et le stress dépendant du contexte ont affecté le transfert d'énergie au niveau des copépodes (*M. littorale* a perdu la moitié de sa teneur énergétique en se nourrissant de diatomées cultivées sous les deux formes de stress chimique). Le stress sélectif, provoquant des changements dans la structure de la communauté vers la domination par des espèces à faible contribution fonctionnelle, a été identifié comme une menace plus puissante pour le fonctionnement que tout effet de stress direct sur la contribution fonctionnelle de l'espèce. Le contenu énergétique des copépodes dépendait de celui des diatomées ingérées. Cela souligne l'importance de la qualité alimentaire comme facteur-clé de transfert d'énergie des producteurs primaires aux consommateurs.

Dans le chapitre 6, un cadre conceptuel a été développé pour présenter les trois principaux effets déterminant l'effet du stress sur la relation entre la biodiversité et le fonctionnement des écosystèmes. 1. **Le stress sélectif**, ciblant les espèces fonctionnellement les plus importantes, augmente avec les différences dans la contribution fonctionnelle des espèces et peut être prévu par les traits de réponse et traits d'effet des espèces. 2. **Le stress physiologique** influence le fonctionnement de l'écosystème en modifiant directement la contribution fonctionnelle des espèces. 3. **La complémentarité**, stimulée par l'activation de mécanismes facilitateurs sous stress, peut permettre aux producteurs primaires de maintenir leur contribution fonctionnelle et de limiter les pertes de fonctionnement des écosystèmes. En mettant en évidence les trois principaux effets modulant la relation entre la diversité et le fonctionnement, cette thèse propose un cadre visant une meilleure intégration des facteurs de stress anthropiques dans la recherche sur la biodiversité et le fonctionnement des écosystèmes.

Chapter 1: General Introduction

1.1. Biodiversity and ecosystem functioning

Over the past century, Earth has experienced an unprecedented and widespread biodiversity turnover, and current predictions indicate that the rates of biodiversity change will continue to rise in the near future (Sala et al. 2000, Pereira et al. 2010, Barnosky et al. 2011, Dornelas et al. 2014, Ceballos et al. 2015, Gonzalez et al. 2016). Human activities have been the main reason for the ongoing extinctions and compositional turnover and will continue to extensively alter biodiversity worldwide (Pimm et al. 1995, Sala et al. 2000, Chapin et al. 2000, Bellard et al. 2012, McGill et al. 2015). Biodiversity has long been treated as a matter of 'pure ecology', which sees biodiversity as a culmination of population and community ecological processes, and changes in biodiversity have sparked mainly aesthetic and ethical concerns (Yarmin 1995, Martín-López et al. 2007, Naeem et al. 2009). This conventional perspective of biodiversity has been revolutionized by a question that had rarely been asked by ecology texts: What is the significance of biodiversity to human wellbeing (Naeem et al. 2009)? This question broadened the conventional perspective of biodiversity as a pure result of ecological processes towards the idea of biodiversity as driver of ecosystem processes, which underpin the ecosystem goods and services that ultimately determine human well-being (Loreau et al. 2001b, Hooper et al. 2005, Srivastava and Vellend 2005, Cardinale et al. 2012, Naeem et al. 2012).

1.1.1. From community and ecosystem ecology to biodiversity-ecosystem functioning research

Until the end of the 20th century, the inclusion of biodiversity into ecosystem science has not been a focus in ecological research, which has traditionally been divided into the separate disciplines of community ecology and ecosystem ecology (Loreau 2000, Loreau et al. 2001a). The former focused on how biodiversity and community composition are governed by biotic interactions (e.g. competition, predation, mutualism) and abiotic processes such as disturbance and climate. The latter centred on the properties within ecosystems, such as the rates, dynamics or stability of energy flow or nutrient cycling (Loreau et al. 2001a). In the early 1990s however, the separation of community and ecosystem ecology was overcome when researchers started investigating the impact of biota on ecosystem properties (Lubchenco et al. 1991, Chapin et al. 1992, Hobbs 1992).

Vitousek and Hooper (1993) formally merged community and ecosystem research and proposed a common positive relationship between biodiversity and ecosystem functioning (hereafter BEF). BEF research rapidly received widespread attention, and the first BEF experiments investigating the effects of biodiversity on plant productivity in the mid-1990s (Naeem et al. 1994, Tilman and

Downing 1994, Tilman et al. 1996) were followed by in a dramatic increase of empirical and theoretical studies (Fig. 1.1).

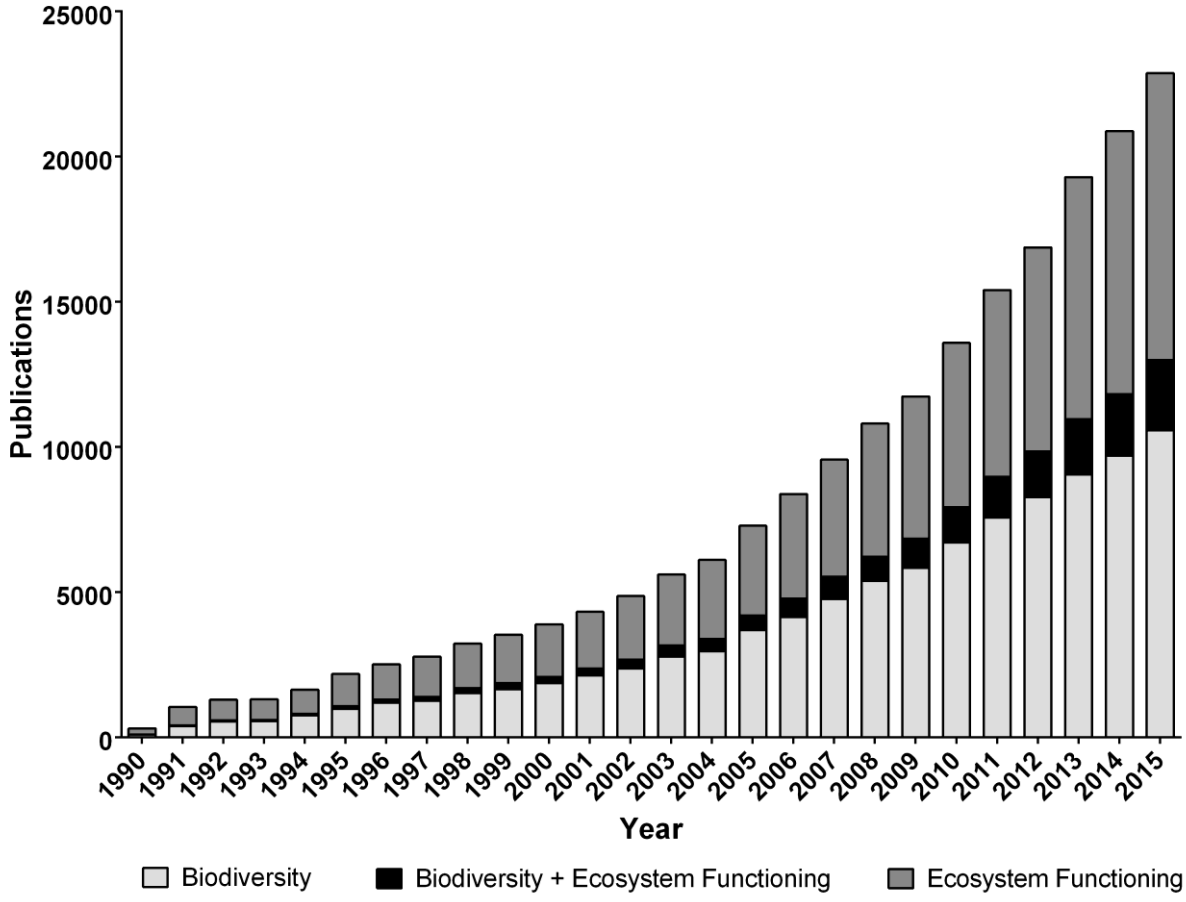


Fig. 1.1: The emerging synthesis of biodiversity and ecosystem functioning research. Results represent publications that included either biodiversity (B), ecosystem functioning (EF) or both (BEF, black fill in the centre of bars) in their titles, keywords or abstracts. Note the steep increase in biodiversity research, and the increasing proportion of studies linking biodiversity with ecosystem functioning. Data were obtained from the *ISI Web of Knowledge* using the 'advanced search' tool and the *Science Citation Index Expanded* database on 29th July 2016. The general search terms were TS=('biodiversity' OR 'species diversity' OR 'species richness' OR 'evenness'*) for publications on biodiversity, TS=('ecosystem function' OR 'ecosystem functioning' OR 'ecosystem process' OR 'ecosystem') for publications on ecosystem functioning, and TS=('biodiversity' OR 'species diversity' OR 'species richness' OR evenness) AND TS=('ecosystem function' OR 'ecosystem functioning' OR 'ecosystem process' OR 'ecosystem') for publications including both terms. The search included the keywords, titles and abstracts of all articles in all languages between 1990 and 2015.

Over the last decade, BEF research has provided an extensive amount of hypotheses and case studies, unraveled the core mechanisms (see 1.6) driving the BEF relation, and allowed for an unambiguous interpretation of the biodiversity effect on ecosystem functioning (Schläpfer and Schmid 1999, Loreau and Hector 2001, Loreau et al. 2001b, 2002, Fox 2005, Hooper et al. 2005, 2012, Cardinale et al. 2011, Tilman et al. 2014). At present, the empirical evidence from more than

a thousand BEF studies indicates a positive effect of biodiversity on ecosystem processes such as biomass production, nutrient flux and decomposition (Worm et al. 2006, Balvanera et al. 2006, Cardinale et al. 2006, 2007, 2012, Stachowicz et al. 2007, Finkel et al. 2010, Quijas et al. 2010, Hooper et al. 2012, Tilman et al. 2014, Gamfeldt et al. 2015, Hautier et al. 2015). Although biodiversity effects were by no means universal and the slope of the BEF relation has shown considerable variation, there appears to be a striking level in consistency of biodiversity effects across various organism groups such as microbes, plants and predators, among trophic levels and across the different types ecosystems that have been studied (Balvanera et al. 2006, Cardinale et al. 2006, 2011, Gamfeldt et al. 2008, 2015, Wagg et al. 2014).

1.1.2. Defining biodiversity, ecosystem functioning and ecosystem processes

The term biodiversity encompasses a broad spectrum of biotic scales, which include genetic variation within species, the numbers and relative abundances at the species level, and the diversity of ecosystems on the planet (Purvis and Hector 2000, Mooney 2002, Hooper et al. 2005). This broad scale is reflected in the biodiversity synthesis of the Millennium Ecosystem Assessment (2005), which defines biodiversity as 'the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.' BEF research commonly focuses on biodiversity at the species level (Loreau et al. 2002, Stachowicz et al. 2007), and this thesis consequently describes diversity in terms of both the number of species (species richness) and their relative abundance (species evenness).

In all ecosystems, ecosystem processes performed by biota deliver multiple ecosystem functions that contribute to the delivery of ecosystem services (Snelgrove et al. 2014). Following the definitions by Snelgrove et al. (2014), the *ecosystem processes* performed by biota (e.g. in this work the production of extracellular polymers or fatty acids) are the precursors to ecosystem functions. *Ecosystem functions* (in this work e.g. sediment stabilisation or energy transfer) are defined as changes in energy and matter over time and space through biological activity. These functions sustain *ecosystem services*, i.e. the benefits humans obtain from ecosystems, for instance shoreline stability or food production (Snelgrove et al. 2014). As distinct from BEF research, which has linked biodiversity and ecosystem functioning through experimental work and mathematical theory, studies on biodiversity and ecosystem services concentrate on larger-scale patterns across landscapes, with the economic and cultural evaluation of biodiversity changes as a focal point (Cardinale et al. 2012, Mace et al. 2012), which is however beyond the scope of this thesis. Although in BEF research the interchangeable use of the terms ecosystem process and ecosystem function is widely accepted (Wallace et al. 2007), this thesis uses both terms separately to acknowledge that the ecosystem functions addressed in this work are driven by a multitude of

processes. The term ecosystem function is used when discussing general concepts of BEF research, the term ecosystem process is used when discussing the specific processes carried out by a species or community. Italicised terms are defined in a box after the paragraph where they were first mentioned.

Ecosystem process	Processes performed by biota which contribute to ecosystem functions
Ecosystem function	Changes in energy and matter over time and space through biological activity
Ecosystem services	The benefits humans obtain from ecosystems

1.1.3. Evenness and Multifunctionality

Most BEF research to date focused on individual ecosystem processes in isolation, with the biomass production of primary producers being the most common functional endpoint (Balvanera et al. 2006, Gamfeldt et al. 2013). Whilst biomass production is vital for delivering a large range of ecosystem services (Imhoff et al. 2004), the contribution of biological communities to decomposition, nutrient cycling, habitat structuring or trophic energy flow are also of key importance for ecosystem functioning (Müller-Navarra et al. 2000, Gamfeldt et al. 2005, Rabaut et al. 2007, Manzoni et al. 2008, Godbold et al. 2009). If diversity effects are not quantified for a larger set of ecosystem functions, BEF research will only partially capture an ecosystem's response to biodiversity loss, since functions other than primary production respond differently to biodiversity change and diversity effects on ecosystem multifunctionality are larger than diversity effects on single functions (Hector and Bagchi 2007, Jiang et al. 2008, Gamfeldt et al. 2013, Lefcheck et al. 2015).

Moreover, most BEF studies have chosen *species richness* as measure of diversity, and have held the relative abundance of species constant across richness treatments (Wilsey and Polley 2004, Hillebrand et al. 2008, Isbell et al. 2009). Until the end of the last decade, 90% of BEF experiments were based on manipulations of species richness, whereas less than 2.5% examined changes in relative species abundances (i.e. *species evenness*, Balvanera et al. 2006, Hillebrand and Matthiessen 2009). However, the impact of a species on ecosystem functioning depends not only on whether it is present in an ecosystem, but also on how abundant it is (Díaz et al. 2003). If the dominant species contributes more to ecosystem functioning than the mean of the community, functioning will increase, and vice versa if the dominant species performs below average (Hillebrand et al. 2008). Human activities do not only alter the number, but also the relative

abundance of species (Hillebrand et al. 2008). More importantly, evenness commonly responds more sensitively to environmental change than species richness, and changes in evenness can affect ecosystem functioning long before species are actually threatened by extinction (Chapin et al. 2000, Hillebrand et al. 2008). Thus, the distribution and relative abundance of species within a community might be more important for its contribution to ecosystem functioning than the number of species (Norberg 2004, Hillebrand et al. 2008).

Species richness	The number of species in a community or ecosystem
Species evenness	The relative abundance of species in a community or ecosystem

1.2. Biodiversity and ecosystem functioning in marine systems

Marine ecosystems are of particular interest to conservation science due to their tremendous biodiversity, with a significant number of marine species not yet described, and due to their global significance for the storage and cycling of materials, nutrients, and energy flow (Snelgrove 1999, Covich et al. 2004). Coastal, estuarine and freshwater ecosystems are among the most impaired parts of the biosphere, experiencing some of the highest rates of species loss, but host a disproportionately large fraction of productivity, providing essential services for human well-being (Malmqvist and Rundle 2002, Covich et al. 2004, Gamfeldt et al. 2015, Turner and Schaafsma 2015). However, most of the early research on the effects of biodiversity on ecosystem functioning has been focussing on terrestrial ecosystems, particularly grasslands, with producer biomass as the predominant functional endpoint (Balvanera et al. 2006, Jiang et al. 2008). Marine and freshwater systems on the other hand have remained a 'niche product' in early BEF research, and conservationists stressed the need to test if conclusions derived from terrestrial experiments also applied to aquatic ecosystems (Covich et al. 2004, Giller et al. 2004). During the last decade, a number of groundbreaking studies responded to these needs and investigated the effects of biodiversity loss on the functioning of marine and particularly coastal systems (e.g. Emmerson et al. 2001, Bolam et al. 2002, Solan et al. 2004, Bracken et al. 2008, Godbold et al. 2009, Vanelslander et al. 2009, Davies et al. 2011, Gustafsson and Boström 2011, Baert et al. 2016). While the majority of BEF research is still based on studies in terrestrial systems (60 % of all BEF contributions as identified by Solan et al. 2009), marine systems are increasingly well represented (11%, the remaining contributions being 13% freshwater studies and 17% generic reviews). By now, the empirical evidence obtained in recent marine studies suggests that biodiversity tends to enhance ecosystem functioning not only in terrestrial, but also in marine habitats (Gamfeldt et al. 2015).

583 **1.3. Anthropogenic stress**

584 In physiological and medical research, the term stress is most generally defined as a condition that
585 causes an organism to shift away from its homeostatic balance (Cannon 1926, Goldstein and Kopin
586 2007, Neumann-Lee 2016). In plant ecology, definitions of stress primarily focus on the altered
587 physiological state, impairing the performance of vital functions (Gaspar et al. 2002), with stress
588 being defined as changes in environmental conditions which have the capacity to cause body
589 injury, disease or aberrant physiology (Gaspar et al. 2002), and affect a plant's metabolism, growth
590 or development (Lichtenthaler 1996). An unfavourable condition which is caused by human
591 activities, can reduce plant vitality and causes damage to plants is termed *anthropogenic stress*
592 (Lichtenthaler 1998). In a biodiversity context, the term anthropogenic stress is commonly used
593 to describe human-induced changes in environmental conditions which lead to species loss or
594 altered community structure (e.g. Wickham et al. 1997, Carlisle and Clements 2005, Halpern et al.
595 2007, Johnston and Roberts 2009, McMahon et al. 2012, Malaj et al. 2014, Neumann-Lee 2016).
596 By merging the physiology-focused and richness-focused stress definitions from plant ecology
597 and biodiversity research respectively, anthropogenic stress can be defined as a change in
598 environmental conditions which is caused by human activities and affects organismal physiology
599 and / or community structure.

600 Anthropogenic stress is among the driving forces behind the ongoing biodiversity losses (Lotze et
601 al. 2006, Halpern et al. 2008, Geiger et al. 2010). Among the many anthropogenic impacts on
602 natural ecosystems, chemical pollution is regarded as one of the most potent threats to aquatic
603 biodiversity (Wilcove and Master 2005). In the EU alone there are more than 100 000 registered
604 chemicals (EU 2001), many of which have not been tested for adverse biological effects. Global
605 chemical production is projected to double over the next quarter-century, rapidly outpacing the
606 rate of population growth, and many of these chemicals ultimately enter the environment
607 (Schwarzman and Wilson 2009). The list of chemicals eventually found in coastal ecosystems
608 includes industrial, domestic and agricultural nutrients, pesticides, road run-off, personal care
609 products, disinfectants and pharmaceuticals (European Marine Board 2013), amongst which
610 pesticides and their residues are reported to be among the most devastating agents for aquatic
611 ecosystems, affecting all levels of the food chain from the lowest to the top level (Islam and Tanaka
612 2004). Chemical pollution is jeopardizing biodiversity on a global scale (Wilcove and Master 2005,
613 Backhaus et al. 2012, Malaj et al. 2014), but the percentages of ecological studies addressing
614 pollution do not correspond at all to its ranked importance as a driver for biodiversity loss (Lawler
615 et al. 2006). This underrepresentation of anthropogenic stress in studies testing the effects of
616 biodiversity loss on ecosystem functioning has raised critical questions whether the patterns and

diversity-functioning relations obtained in classic BEF experiments reflect those induced by anthropogenic activities (Naeem 2008, Fugère et al. 2012, O'Connor et al. 2015).

Anthropogenic stress	A change in environmental conditions which is caused by human activities and affects organismal physiology and / or community structure

1.4. Incorporating anthropogenic stress into BEF research

The overwhelming majority of BEF experiments to date have manipulated biodiversity in subsets of species under controlled conditions, which enabled scientists to eliminate functional effects by confounding factors other than biodiversity, and thus allowed an unambiguous interpretation of the BEF relation (Loreau et al. 2001b, Naeem and Wright 2003, Díaz et al. 2003, Srivastava et al. 2004, Hooper et al. 2005, Cardinale et al. 2006, 2012, Tilman et al. 2014). The high degree of control required in these experiments however implied that the environmental complexity and the anthropogenic drivers that shape BEF relations under global change were largely neglected (Zavaleta and Hulvey 2004, Srivastava and Vellend 2005, Schläpfer et al. 2005, Bracken et al. 2008, Reich et al. 2012, De Laender et al. 2016). The plethora of anthropogenic drivers causing the ongoing biodiversity changes in nature however made it unclear whether the observed BEF patterns will hold for realistic extinction scenarios as induced by anthropogenic change, over multiple trophic levels, and across different types of ecosystems (Giller et al. 2004, Srivastava and Vellend 2005, Raffaelli 2006, Duffy et al. 2007, Hillebrand and Matthiessen 2009, Gamfeldt et al. 2013). After the first phase in BEF research had provided the empirical evidence on the direction and the effects underpinning the diversity-functioning relation, the incorporation of realistic biodiversity loss and trophic complexity were thus identified as key challenges for the next generation of BEF research (Hooper et al. 2005, Srivastava and Vellend 2005, Cardinale et al. 2012).

1.4.1. The classic BEF design

After early BEF experiments (Naeem et al. 1994, Tilman and Downing 1994) were criticized for confounding species number with species composition, BEF research soon adopted the principle of using random species assemblages to evaluate the functional consequences of changing biodiversity (McGrady-Steed et al. 1997, Hector et al. 1999, Naeem 1999, Tilman et al. 2001, Downing and Leibold 2002). To date, most BEF research has involved experiments in which diversity is varied through random draws from a species pool (Srivastava and Vellend 2005,

Cardinale et al. 2012, Eisenhauer et al. 2016, Wardle 2016). Random designs have considerably advanced BEF research, by allowing to unequivocally attribute changes in functioning to species richness, and by providing insights into the key mechanisms by which more diverse communities can result in enhanced functioning (Loreau and Hector 2001, Cardinale et al. 2006, Eisenhauer et al. 2016). However, the classic design of random species loss involves three implicit limitations, which are addressed in the following three sections.

1.4.2. Selective stress

First, random species loss implies that the likelihood to be removed from the system is the same for all species, regardless of their extinction-proneness in nature, an assumption which soon drew criticism (Ives and Cardinale 2004, Giller et al. 2004). While it is impractical for experimental studies to mimic all the factors driving species loss in nature, it is clearly an oversimplification to accept random extinction as a representative scenario of species loss (Giller et al. 2004). Anthropogenic stress is likely to cause non-random sequences of biodiversity loss, depending on differences in the species' physiology, morphology, trophic position or habitat specialization, which determine their sensitivity to different stressors (Jonsson et al. 2002, Duffy 2003, Ives and Cardinale 2004, O'Connor and Crowe 2005, Bracken et al. 2008). Stress has therefore been termed *selective stress*, if it disfavours some species more than others (Wittebolle et al. 2009). Biodiversity loss driven by selective stress can cause more rapid in ecosystem functioning compared to random loss scenarios, when the species contributing most to functioning are least stress-tolerant (Fig. 1.2.1, Larsen et al. 2005, McIntyre et al. 2007, Bracken et al. 2008, 2012). Conversely, if a stressor mainly targets species contributing little to functioning, the impact of selective stress on ecosystem functioning will be limited (Fig. 1.2.2, Solan et al. 2004, Radchuk et al. 2016). If a stressor does not alter community composition (Fig. 1.2.3, Plumley and Davis 1980, Griffiths et al. 2003, Kearns et al. 2016), effects on functioning will be driven by direct stress effects on the species' physiology (see 1.4.3) or changes in species interactions (see 1.6). Selective shifts in primary producer composition can furthermore lead to knock-on effects across trophic level boundaries, impacting ecosystem functioning at the consumer level (Bracken and Low 2012, Hogsden and Harding 2012, but see 1.7). The contrasting scenarios of selective biodiversity loss have raised questions about how useful inferences from randomly assembled communities will be for conservation efforts, and has led to calls for experimental designs that remove the most vulnerable species first (Duffy 2003, Raffaelli 2004, Ball et al. 2008, Naeem 2008, Tilman et al. 2014, Wardle 2016).

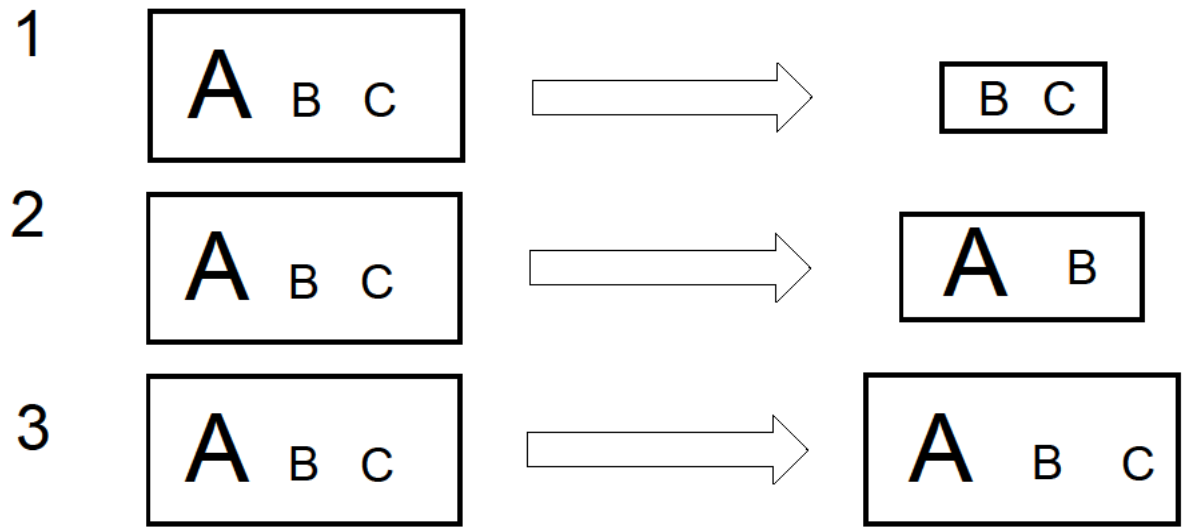


Fig. 1.2: Potential effects of selective stress on ecosystem functioning. For the sake of simplicity, this scheme uses communities with only three species (A, B and C). The font size of A, B and C indicates the species' functional contribution. Communities are marked by black squares whose size denotes the community's functional contribution. Communities on the left-hand side of panels 1 to 3 are not exposed to stress, communities on the right-hand side of are exposed to stress. In panel 1, selective stress removes the species contributing most to functioning (species A), resulting in a disproportionate loss of functioning. In panel 2, selective stress removes species B, but the functionally most important species (species A) persists under stress, resulting in a limited loss of functioning. In panel 3, stress does not alter community composition. Potential stress-induced changes in ecosystem functioning would then solely be driven by direct stress effects on the species' contribution to functioning (see Fig. 1.3) or changes in species interactions (see Fig. 1.4).

1.4.3. Physiological stress

Second, the same stressors that indirectly alter ecosystem functioning through selective changes in biodiversity can also directly affect the physiology of the surviving species, and thus their contribution to ecosystem functioning. Direct exposure to anthropogenic stressors is however rarely included into the design of BEF studies (McMahon et al. 2012, but see Reusch et al. 2005, Wittebolle et al. 2009, McMahon et al. 2012, Roger et al. 2012, Baert et al. 2016, Radchuk et al. 2016). This means that even when diversity gradients are established according to the species' vulnerability in nature, the functional rates of the surviving 'tolerant' species are commonly assumed to remain unaffected. However, stress tolerance may come at a physiological cost, e.g. acclimation by allocating resources from growth to survival pathways (Schimel et al. 2007), and this '*physiological stress*' can cause species to contribute differently to a given ecosystem processes

than in unstressed conditions (Relyea and Hoverman 2006, Schimel et al. 2007, De Laender et al. 2010). This is at least the case for chemical stress, which commonly causes sublethal adverse effects on the surviving species, reducing their contribution to ecosystem functioning (Fig. 1.3.1, McWilliam and Baird 2002, Fleeger et al. 2003, Rohr et al. 2006, Liebig et al. 2008). Conversely, low doses of stress can also trigger a hormetic response (Calabrese and Baldwin 1998, Calabrese 2008), thus increasing organisms' contribution to a particular ecosystem processes (Fig. 1.3.2, Bartell 2000). If organismal physiology is not affected (Fig. 1.3.3), potential changes in ecosystem functioning will be driven by selective changes in community composition (see 1.4.2) or changes in species interactions (see 1.6). Even when explicitly including exposure to anthropogenic stressors, common BEF designs cannot separate the direct functional impact of a stressor via effects on organismal physiology from its indirect effects that are mediated by selective changes in biodiversity (Relyea and Hoverman 2006, Radchuk et al. 2016). Disentangling direct stress effects at the physiological level and from indirect effects at the community level thus represents a major challenge to determine functioning in stressed communities (Relyea and Hoverman 2006, Schimel et al. 2007).

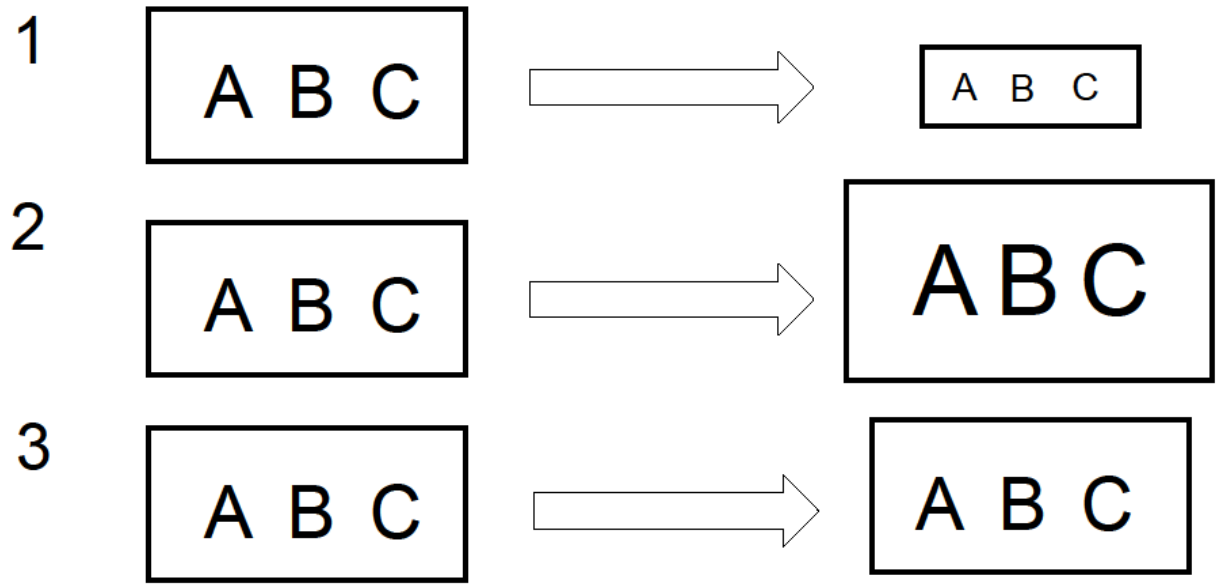


Fig. 1.3: Potential effects of physiological stress on ecosystem functioning. For the sake of simplicity, this scheme uses communities with only three species (A, B and C). The font size of A, B and C indicates the species' functional contribution. Communities are marked by black squares whose size denotes the community's functional contribution. Communities on the left-hand side of panels 1 to 3 are not exposed to stress, communities on the right-hand side of are exposed to stress. In panel 1, physiological stress reduces the species' contribution to ecosystem functioning. For the sake of simplicity, it is assumed the three species contribute equally to functioning, with each species' functional contribution altered to the same extent by stress. In panel 2, stress stimulates the species' contribution to a given ecosystem process (hormesis). In panel 3, the species' functional contribution is not affected by stress. Stress-induced changes in ecosystem functioning would in this case only be driven by selective changes in community composition (see Fig. 1.2) or changes in species interactions (see Fig. 1.4).

730

731 **1.4.4. Functional contribution**

732 Third, random species loss holds the implicit assumption that all species contribute in a more or
733 less similar way to ecosystem functioning, and that what matters is mainly the number, rather
734 than the characteristics, of species added or lost from a system (Díaz et al. 2003). The contribution
735 to functioning is however driven by the species' traits (but see 1.5), and the above assumption
736 only holds in a scenario of high effective trait dimensionality, i.e. if functioning is driven by
737 multiple uncorrelated traits. When effective trait dimensionality is low, i.e. functioning is driven
738 by correlated traits or few traits (regardless of correlation), the effects of species richness will be
739 limited and functioning will depend mainly on community composition and species identity
740 (Petchey and Gaston 2002a, 2002b, Petchey et al. 2009). There is indeed mounting evidence that
741 often one or a few particular species have a disproportionate influence on ecosystem properties
742 (Power et al. 1996, Grime 1998, Emmerson et al. 2001, Bolam et al. 2002, Waldbusser et al. 2004,
743 Solan et al. 2004, Bruno et al. 2006, Cardinale et al. 2006). The few studies that investigated
744 selective biodiversity loss caused by anthropogenic disturbance found that anthropogenic
745 stressors can on the one hand have limited effects on functioning despite causing significant
746 diversity loss, as long as the most abundant species are not affected (Smith and Knapp 2003, Solan
747 et al. 2004, Radchuk et al. 2016). On the other hand, BEF studies found a disproportionate loss of
748 functioning when selective stress targeted the species that contributed most to ecosystem
749 functioning (Larsen et al. 2005, McIntyre et al. 2007). Integrating information on the *functional*
750 *contribution* of a species within scenarios of selective biodiversity loss is thus necessary to
751 successfully predict BEF relationships under stress (Hillebrand and Matthiessen 2009).

752 These three limitations do not devaluate the findings obtained in classic BEF studies which were
753 based on random manipulations of richness under constant experimental conditions. Far from
754 that, in the absence of detailed predictions, these early studies provided a general expectation on
755 how diversity affects ecosystem functioning and unravelled the fundamental mechanisms driving
756 the BEF relations (Hector et al. 2001, Loreau and Hector 2001, Srivastava and Vellend 2005).
757 However, after the incorporation of selective, non-random biodiversity loss was identified as a
758 key challenge for future BEF research, scientists were facing the delicate task to connect the
759 extinction vulnerability of individual species with their contribution to ecosystem functioning
760 (Hooper et al. 2005, Cardinale et al. 2012). The results of random-loss BEF experiments could
761 most easily be used to predict the effects of biodiversity loss when this loss naturally occurs
762 independently of species traits (Srivastava 2002, Lepš 2004, Srivastava and Vellend 2005).
763 However, as discussed above, anthropogenic stress is causing a plethora of non-random scenarios

764 of biodiversity loss, where species are lost according to their sensitivity to a given stressor
765 (McKinney 1997, Srivastava and Vellend 2005, Larsen et al. 2005, Rubach et al. 2012, Malaj et al.
766 2014). This species-specific sensitivity is correlated with the species' biological traits, which can
767 cause organisms to be either sensitive or tolerant to different types of stress (Lavorel and Garnier
768 2002, Solan et al. 2004, Buchwalter et al. 2008, Van den Brink et al. 2011). In their comprehensive
769 review on the future challenges of BEF research, Hooper et al. (2005) thus described 'the
770 covariance between traits affecting extinction and those affecting ecosystem processes' as 'the
771 first critical issue for predicting the consequences of non-random biodiversity loss'.

Functional contribution	The contribution of a species to a given ecosystem process
Physiological stress	A change in environmental conditions which affects an ecosystem process by altering the species' functional contribution
Selective stress	A change in environmental conditions which affects an ecosystem process by selectively targeting species with a high or low functional contribution

772

773 **1.5. Response and effect traits**

774 The concepts and definitions of 'traits' have varied over time and ecological disciplines (Violle et
775 al. 2007), but BEF research has mainly considered traits as functional traits, which are defined as
776 morphological, physiological or phenological characteristics of an organism, measurable at the
777 individual level, with trait values showing interindividual variation and affecting organismal
778 performance (Violle et al. 2007, Hillebrand and Matthiessen 2009). As such, functional traits are
779 the pillar for the concept of 'functional diversity'. Functional diversity can be described as the
780 diversity of distinct functional groups, in which species are classified according to their traits, or
781 as continuous gradients of different traits, which both allow to predict the changes in ecosystem
782 processes based on changes in community and thus trait composition (Diaz and Cabido 2001,
783 Petchey and Gaston 2006, Griffin et al. 2009, Hillebrand and Matthiessen 2009). As such,
784 functional diversity, together with the above-mentioned species richness and evenness, rapidly
785 emerged as third main biodiversity endpoint in BEF research (Balvanera et al. 2006).

786 Suding et al. (2008) and Hillebrand and Matthiessen (2009) introduced a new trait concept into
787 BEF research, which connects *response traits* (traits that predict species abundance in responses
788 to environmental change) and *effect traits* (traits that reflect the effects of a species on ecosystem
789 processes). This concept corresponded to the need formulated by Hooper et al. (2005), to predict

790 the consequences of selective biodiversity loss by correlating traits predicting extinction with
791 those affecting ecosystem processes. If response traits and effect traits are correlated, i.e. the
792 species contributing most to functioning are also the ones most likely to be lost in response to
793 anthropogenic disturbance, selective biodiversity loss will lead to a disproportionate decline in
794 functioning relative to random biodiversity loss. If response and effect traits are uncorrelated,
795 some functionally efficient species are able to persist and maintain ecosystem functioning
796 (Lavorel and Garnier 2002, Suding et al. 2008, Hillebrand and Matthiessen 2009). Toxic chemicals
797 however do not only alter the abundance of species, but also their functional contribution
798 (McWilliam and Baird 2002, Fleeger et al. 2003, Relyea and Hoverman 2006; Schimel et al. 2007;
799 De Laender et al. 2010, but see 1.4.3), by altering the expression of effect traits relevant to
800 ecosystem functioning (Relyea and Hoverman 2006, Rohr et al. 2006). It remains largely
801 unexplored if response traits can predict not only the numerical stress response of species (i.e.
802 changes in abundance, hereafter numerical stress response), but also the functional stress
803 response (i.e. changes in effect traits determining if species can maintain their functional
804 contribution). When the numerical and functional stress response are positively related to the
805 same set of response traits, a community decimated by species loss could still be expected to
806 retain most of its functioning, if numerically tolerant species maintain their contribution to
807 ecosystem functioning. Alternatively, stress exposure could lead to a disproportionate reduction
808 in functioning if numerically tolerant species, while still being able to grow, would lose most of
809 their functional contribution.

810 The use of response traits as predictors of sensitivity to toxic substances has been increasingly
811 advocated in ecotoxicology, as a mechanistic alternative to the otherwise empirical approach of
812 estimating sensitivities using species sensitivity distributions (Baird and Van den Brink 2007, Van
813 den Brink et al. 2011). For example, Baird and Van den Brink (2007) showed that only four
814 response traits could predict the sensitivity of 12 species to a large array of chemical pollutants.
815 Subsequently, several pioneering works have shown the capacity of traits to predict species
816 sensitivity to chemical pollutants, linking traits to specific toxic mechanisms, and thus enabling a
817 more deterministic description of the structure of biological communities under chemical stress
818 (Buchwalter et al. 2008, Guénard et al. 2011, Larras et al. 2012, Rubach et al. 2012, Fischer et al.
819 2013, Pomati and Nizzetto 2013). Whilst the use of response traits is well established, the
820 identification of effect traits and the explicit linkage between both types of traits is still rare in BEF
821 research (but see Pakeman 2011, Heuner et al. 2015, Zwart et al. 2015, Lennon and Lehmkuhl
822 2016), and potential changes in effect traits under stress have not yet been explored. An increased
823 focus on correlating response and effect traits should thus enable scientists to predict how

824 selective changes in diversity caused by environmental change will affect the contribution of
825 biological communities to ecosystem functioning.

Response trait	A trait predicting the abundance of a species or organism group in response to a change in environmental conditions
Effect trait	A trait reflecting the effect of a species or organism group on ecosystem processes

826

827 **1.6. Complementarity and dominance**

828 The identification of complementarity and selection effects have been among the most crucial
829 findings of early BEF research (Loreau and Hector 2001). After more than two decades of BEF
830 research, scientists now widely agree that complementarity and selection are the two key effects
831 which cause diverse communities to perform differently than expected from their monocultures
832 (Hector et al. 2009, Hodapp et al. 2016). The selection effect drives BEF relations through selective
833 processes such as interspecific competition, which can cause dominance (high relative
834 abundance) of species with particular traits. The selection effect can positively or negatively affect
835 a community's contribution to ecosystem functioning, depending on whether the species that
836 ultimately dominate the community have a relatively high or low functional contribution,
837 respectively (Loreau and Hector 2001). In contrast to the competition-driven selection effect,
838 complementarity refers to a class of mechanisms that result in a higher performance of a
839 community than would be expected from the separate performances of each component species
840 (Loreau and Hector 2001). These mechanisms are commonly thought to be facilitation, e.g. if
841 species modify the environment in a way that benefits co-occurring species, and niche
842 partitioning, e.g. different resource requirements among species causing interspecific competition
843 in diverse communities to be lower than intraspecific competition in monocultures (Tilman et al.
844 1997, Loreau and Hector 2001, Duffy et al. 2007, Vanelslander et al. 2009).

845 One limitation of Loreau and Hector's (2001) bipartite partition of the biodiversity effect into
846 selection and complementarity is that it assumes that complementarity is distributed equally
847 across species, i.e. that species mutually benefit each other to the same degree (Hector et al. 2009).
848 However, species with particular traits can perform better than expected in communities either
849 due to competitive replacement of other species, or not at the expense of other species, e.g. by
850 benefitting from complementarity mechanisms without contributing to these mechanisms (the
851 so-called trait-dependent or 'one-way' complementarity, Fox 2005; Hector et al. 2009). The
852 bipartite partition assigns this trait-dependent complementarity to the selection effect, and may

853 therefore over- or underestimate total complementarity, depending in whether species with
854 particular traits (e.g. high or low monoculture biomass, hereafter yield) benefit more or less from
855 complementarity (Fox 2005; Hector et al. 2009). Therefore, Fox (2005) proposed a tripartite
856 model, that partitions the biodiversity effect on ecosystem functioning into *dominance*, *trait-*
857 *dependent complementarity* and *trait-independent complementarity* effects. The dominance effect
858 occurs when species with particular traits dominate communities at the expense of other species.
859 Depending on whether communities are dominated by high- or low-yield species, the dominance
860 effect will be positive or negative. Trait-dependent or one-way complementarity occurs when
861 species with particular traits perform better in community than in monoculture, but not at the
862 expense of other species. Trait-independent or two-way complementarity occurs when species
863 perform better in community, independently of their traits and not at the expense of other species.
864 Trait-independent complementarity is thus analogous to complementarity as defined by Loreau
865 and Hector (2001). To date, there have been few explicit comparisons of the bipartite and
866 tripartite partitioning methods (Hector et al. 2009), but where tested, the contribution of trait-
867 dependent complementarity to the total biodiversity effect was minor (Hector et al. 2009, Karlson
868 et al. 2010, Schmidtke et al. 2010, Fernandes et al. 2011, Long et al. 2013, Stachova et al. 2013,
869 Siebenkäs et al. 2016). Thus, using the bi- or tripartite partition should produce similar results on
870 the relative importance of dominance / selection and complementarity effects.

871 More importantly, experimental BEF studies keep producing contrasting results on the relative
872 contributions of dominance and complementarity effects, leading to an active debate on which
873 principal effect drives the diversity-functioning relation (Huston et al. 2000, Cardinale et al. 2006,
874 Fargione et al. 2007, Wang et al. 2013, Hodapp et al. 2016). The bottleneck which is limiting the
875 experimental quantification of complementarity and dominance effects, is that in order to
876 unequivocally determine the contribution of either effect to observed BEF relationships,
877 information is required on the yield of every species when grown in community and in
878 monoculture. This information is however largely unavailable (Hector et al. 2009), and as a result
879 complementarity and dominance underpinning observed BEF relationships are largely
880 interpreted indirectly from existing patterns, rather than from direct experimental tests
881 (Cardinale et al. 2006, Hector et al. 2009).

882 The contribution of dominance and complementarity effects to BEF relations under stress is even
883 less clear. Whilst anthropogenic stressors are increasingly incorporated into the design of BEF
884 experiments, these experiments found contrasting biodiversity effects on functioning in stressed
885 communities: clearly positive effects in Mulder et al. (2001), Solan et al. (2004), Zavaleta and
886 Hulvey (2004), Larsen et al. (2005), Ives and Carpenter (2007), McMahon et al. (2012), Steudel et
887 al. (2012), Wang et al. (2013), and Baert et al. (2016), neutral or even negative diversity effects in

888 Jonsson et al. (2002), Petchey et al. (2002c), Smith and Knapp (2003), Jiang (2007), Fernandes et
889 al. (2011), Roger et al. (2012) and Fugère et al. (2012). However, few of these discrepant diversity-
890 functioning relations could be explicitly traced to changes in dominance and complementarity
891 effects (Fernandes et al. 2011, Fugère et al. 2012, Wang et al. 2013, Baert et al. 2016, Hodapp et
892 al. 2016), since quantifying both effects under stress requires data on the yield of every species in
893 community and monoculture at every stress level. Under stressful conditions, species will become
894 dominant by being inherently tolerant and thus replacing other more sensitive species. This is the
895 selective stress described before (see 1.4.2) and although dominance effects could not be explicitly
896 quantified, the functional contribution of these dominant, stress-tolerant species has been
897 identified as key driver of BEF relations under stress (Petchey et al. 2002c, Solan et al. 2004,
898 Zavaleta and Hulvey 2004, Larsen et al. 2005, Radchuk et al. 2016). Complementarity effects in
899 stressed communities depend on potential changes in species interactions along stress gradients.
900 If stress does not alter the strength of species interactions, the diversity-functioning relation will
901 be driven by direct stress effects on the species' functional contribution or by the replacement of
902 sensitive by stress-tolerant species (see 1.4.1, Baert et al. 2016). An increase in interspecific
903 competition can cause negative complementarity effects which reduce ecosystem functioning
904 under stress (Fig. 1.4.2, Fernandes et al. 2011, Becker et al. 2012). The complementarity effect
905 increases under stress if facilitation or niche partitioning are enhanced (Fig. 1.4.3, Fugère et al.
906 2012; Wang et al. 2013). Direct ecophysiological evidence for interspecific facilitation as driver of
907 complementarity effects is however still limited (Cardinale et al. 2002, Temperton et al. 2007,
908 Vanelslander et al. 2009), and is lacking so far in stressed communities. Determining the relative
909 importance of dominance and complementarity effects as drivers of BEF relations under stress,
910 and connecting both effects to stress tolerance and interactions at the species level, thus remains
911 a largely unresolved task for explaining diversity-functioning relations in stressed communities.

Dominance effect	Species with particular traits dominate communities at the expense of others.
Trait-independent complementarity	Growth in community rather than monoculture increases the functioning of species, independent of their traits and not at the expense of other species.
Trait-dependent complementarity	Growth in community rather than monoculture increases the functioning of species with particular traits, but not at the expense of other species.

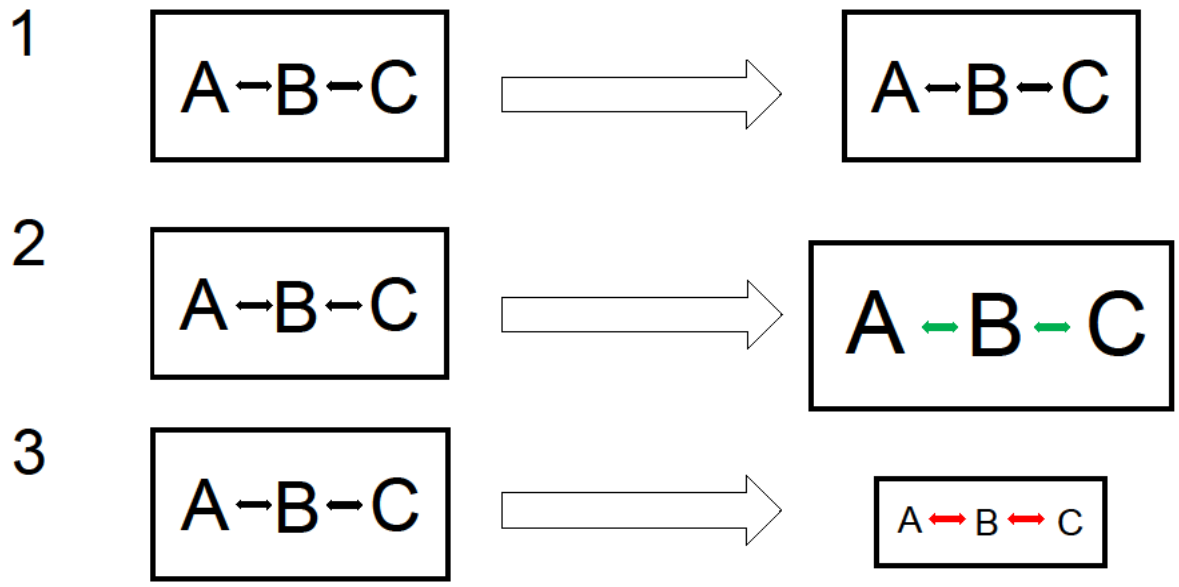


Fig. 1.4: Potential changes in the complementarity effect under stress. For the sake of simplicity, this scheme uses communities with only three species (A, B and C). The font size of A, B and C indicates the species' functional contribution. Communities are marked by black squares whose size denotes the community's functional contribution. Communities on the left-hand side of panels 1 to 3 are not exposed to stress, communities on the right-hand side of are exposed to stress. Arrows between A, B and C denote species interactions. In panel 1, species interactions are not altered by stress, and the complementarity effect stays constant. The diversity-functioning relation under stress is driven by the same species interactions as under unstressed conditions. Hence, stress should principally affect the diversity-functioning relation through physiological stress effects (Fig. 1.3), or by selective changes in community composition (Fig. 1.2). In panel 2, species interactions under stress become more positive compared to control conditions (visualised by the green arrows), and the complementarity effect increases under stress. The underlying ecological mechanisms are an increase in niche partitioning or in facilitative interactions, augmenting the species' functional contribution when exposed to a stressor in community. In panel 3, species interactions under stress become more negative compared to control conditions (visualised by the red arrows) and the complementarity effect decreases under stress due to an increase in interspecific competition, reducing the contribution of stressed communities to ecosystem functioning.

1.7. Trophic complexity

1.7.1. Horizontal and vertical biodiversity effects

BEF research has been dominated by experiments focussing on a single trophic level, mostly primary producers in terrestrial grasslands (Petchey et al. 2004, Srivastava et al. 2004, O'Connor and Crowe 2005, Raffaelli 2006, Scherber et al. 2010). Biodiversity loss can act horizontally, i.e. affect the abundance, biomass or resource use at the same trophic level where biodiversity is lost (Cardinale et al. 2006, Duffy et al. 2007, Scherber et al. 2010). However, horizontal species loss may also have vertical consequences, by affecting other trophic levels, organism groups and processes (Cardinale et al. 2006; Duffy et al. 2007; Scherber et al. 2010). Besides the incorporation of realistic non-random species loss, the addition of a vertical dimension of biodiversity was thus soon identified as a major task for BEF research (Hooper et al. 2005, Srivastava and Vellend 2005, Duffy et al. 2007, Reiss et al. 2009, Scherber et al. 2010, McMahon et al. 2012).

Although the single trophic level approach has advanced BEF research considerably, it has a number of limitations (Raffaelli 2006, Scherber et al. 2010). For instance, complementarity and dominance effects have been identified as drivers of BEF relations within trophic levels, but these approaches cannot be used to test biodiversity effects across multiple trophic levels, and it is unclear if the same effects occur in multitrophic experiments (Fox 2006, Raffaelli 2006). Another main reason to adopt a second, vertical perspective in BEF research is the need to recognise that biodiversity loss due to anthropogenic activities usually occurs at all trophic levels, and taxa will differ in their sensitivity to particular loss scenarios (Solan et al. 2004, Raffaelli 2006). Thus, anthropogenic stressors can cause differential biodiversity loss and disparate consequences for functioning across trophic levels (Raffaelli 2006, McMahon et al. 2012). There are likely to be significant feedbacks between the trophic level targeted by a stressor and the higher and lower trophic levels (Raffaelli 2006). If these feedbacks are not recognised, the outcomes of one-dimensional, single trophic experiments will only be of limited value for policy makers interested in the impacts of biodiversity loss (Raffaelli et al. 2002, Raffaelli 2006).

In principle, adopting a two-dimensional approach links the fields of BEF research (focusing on horizontal diversity and ecosystem functioning) and predator-prey and food web ecology (focusing of vertical diversity), which are thematically linked but have remained largely separate research fields (Duffy et al. 2007). A large number of trophic ecology studies has analysed the mechanisms and importance of trophic interactions (e.g. Pace et al. 1999, Chase et al. 2002, Borer et al. 2005, De Troch et al. 2005, 2012b). Most of this research however has yet to be integrated within BEF research and its (so far horizontal) focus on ecosystem processes (Duffy et al. 2007). After the first generation of BEF research had established the generality of the diversity-functioning relation, theoretical and experimental works began to merge the functional effects of diversity and of trophic interactions (e.g. Fox 2004, Petchey et al. 2004, Thebaut and Loreau 2005, Casula et al. 2006). Similar to traditional horizontal BEF approaches, vertical BEF research started in terrestrial environments (Koricheva et al. 2000, Balvanera et al. 2006, Cardinale et al. 2006, Haddad et al. 2009), with vertical BEF efforts in marine systems starting in the second half of the last decade (Gamfeldt et al. 2005, Bruno et al. 2008, Scherber et al. 2010). Recent works found strong correlations between primary producer diversity and ecosystem processes at higher trophic levels, thus confirming the importance of biodiversity in a vertical dimension (Scherber et al. 2010, Lefcheck et al. 2015). These findings also apply to chemical pollutants, which by causing losses of producer diversity, can significantly impact processes at the associated consumer level (Relyea and Hoverman 2006, McMahon et al. 2012). Whilst some of the strongest impacts of biodiversity loss have been found at the plant-animal interface (Scherber et al. 2010, McMahon et al. 2012, Lefcheck et al. 2015), these multitrophic diversity-functioning relationships were less

predictable than those found for single trophic levels (Duffy et al. 2007, Reiss et al. 2009, McMahon et al. 2012).

1.7.2. Diet quality

The overwhelming majority of both BEF and trophic ecology experiments to date have focused on *diet quantity* (i.e. the abundance and availability of food, mostly quantified as producer biomass), which indeed is a critical determinant for food web functioning (Arts and Wainmann 1999, Balvanera et al. 2006, Österblom et al. 2008, Scherber et al. 2010, Guo et al. 2016). However, the recent integration of diet biochemistry with traditional studies of diet quantity has indicated that not only abundance and availability of food items, but also *diet quality* can be of striking importance for the reproduction and growth dynamics of many aquatic animals (Arts and Wainmann 1999, Österblom et al. 2008).

The differences in the biochemical composition of organisms are most pronounced at the plant-animal interface, which features the largest, but also the most variable energy transfer efficiencies in the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, De Troch et al. 2012b, Guo et al. 2016). Compared to vascular plants, algae represent a high quality diet for primary consumers due to their lower C:N and C:P ratios (Brett et al. 2000, Cross et al. 2003, Lau et al. 2005), the lower proportion of indigestible skeletal cellulose components (Kloareg and Quatrano 1988, Choat and Clements 1998, Hu et al 2013), and their high *polyunsaturated fatty-acid (PUFA)* content (Taipale et al. 2013, Guo et al. 2016). Fatty acids represent the densest form of energy in aquatic ecosystems (Parrish 2009), and are therefore crucial for the energy flux from primary producers to higher levels of the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Kainz et al. 2004, Gladyshev et al. 2011). PUFAs are defined as fatty acids with more than one double bond, and are almost exclusively generated by plants (Brett and Müller-Navarra 1997) from de novo synthesis of palmitic acid and further enzymatic elongase and desaturation reactions (Harwood and Guschina 2009, Cagliari et al. 2011, Taipale et al. 2013).

Amongst all algal classes, Cryptophyceae and Bacillariophyceae (diatoms) are considered as diets of excellent quality for aquatic primary consumers, due to their high content of two specific PUFA components, the so-called *essential fatty acids (EFAs)* eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, Dunstan et al. 1993, Brett et al. 2006, Taipale et al. 2013). Conversely, primary producers with a low concentration of EFAs (e.g. cyanobacteria) are of very poor diet quality for aquatic consumers (Brett et al. 2006, Burns et al. 2011, Taipale et al. 2013). Animals (e.g. crustaceans and fish as well as humans) cannot synthesize these EFAs de novo, and therefore need to obtain these molecules from their diet (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Litzow et al. 2006, Parrish 2009). Diet quality in terms of EFA content is a critical

determinant of the growth rate and reproductive success of primary consumers (Müller-Navarra 1995, Müller-Navarra et al. 2000, von Elert 2002, Arendt et al. 2005). Moreover, primary consumers such as copepods cannot maintain their energy content when feeding on EFA-poor algae species (Koski et al. 1998, Werbrouck et al. 2016). Algal diet quality can even be a stronger driver of primary consumer biomass than diet quantity (Gladyshev et al. 2011), and a lack of algal EFA production can reduce food web functioning by causing trophic decoupling, i.e. high primary productivity with little corresponding increases in consumer biomass (Guo et al. 2016). Algal EFA production has thus been described as a crucial determinant of diet quality and energy transfer across the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Litzow et al. 2006, Parrish 2009, Taipale et al. 2013). The large variation in EFA content among and within algal classes thus makes the diversity and composition of aquatic primary producer communities a key factor for food web functioning (Dunstan et al. 1993, Wyckmans et al. 2007, Taipale et al. 2013, Guo et al. 2016).

Algal diet quality is strongly influenced by changes in abiotic conditions, and can be altered in response to light intensity (Wainman et al. 1999, Guschina and Harwood 2006), nutrient regimes (Guschina and Harwood 2009, Sanpera-Calbet et al. 2016) and temperature (Morgan-Kiss et al. 2006, Piepho et al. 2012, Werbrouck et al. 2016). Far less attention has been devoted to the effect of anthropogenic stress on algal diet quality. Due to the large variation in the EFA content of microalgae (Dunstan et al. 1993, Taipale et al. 2013, Guo et al. 2016), selective changes in algal community composition could potentially change the energy supply for higher trophic levels. Moreover, there is limited but compelling evidence that anthropogenic stressors affect algal EFA production through direct physiological effects. For instance, metal pollution can cause an increasing degree of algal fatty acid saturation (thus lowering the proportion of high-energy EFAs) and a general decrease of EFA production (Chia et al. 2013a, 2013b). Similarly, pesticide stress can cause a general decrease of fatty acid synthesis (Weisshaar et al. 1988, El-Sheekh et al. 1994), or reduce the proportion of EFAs within the total fatty acid pool by inhibiting enzymes involved in fatty acid elongation and thus EFA synthesis (Böger et al. 2000). Nonetheless, diet quality has so far not been integrated in trophic diversity-functioning research. As it has become increasingly clear that diet quality, and not quantity, is the more important factor regulating the efficiency of energy flow through aquatic food webs (Müller-Navarra et al. 2000, Torres-Ruiz et al. 2007, Lau et al. 2009, Gladyshev et al. 2011), the biochemical composition of primary producers should thus be considered when analysing multitrophic BEF relations under anthropogenic stress.

Diet quantity	The abundance and availability of a food source, in this thesis quantified as biomass
Diet quality	The quality of a food source, in this thesis quantified as essential fatty acid content
Polyunsaturated fatty acids (PUFAs)	Fatty acid molecules with more than one unsaturated carbon bond (double bond)
Essential fatty acids (EFAs)	PUFAs which are essential to organismal physiology, which animals cannot synthesize and thus have to take up from their diet

1.8. Study system and thesis outline

1.8.1. Study system

This research was conducted in microcosm experiments using benthic diatoms (Heterokontophyta, Bacillariophyceae) as primary producers and harpacticoid copepods (Crustacea, Copepoda, Harpactidoida) as their main consumers, and atrazine and copper as anthropogenic stressors. Diatoms and copepods were collected at the Paulina intertidal area, a muddy intertidal habitat in the Westerschelde estuary (SW Netherlands).

Within these muddy intertidal systems, microalgal assemblages (microphytobenthos) represent the main primary producers (Underwood and Kromkamp 1999, Forster et al. 2006, Tang and Kristensen 2007). Benthic diatoms are the dominant component of the microphytobenthos, and play an important role for a number of ecosystem processes (Underwood and Kromkamp 1999, Forster et al. 2006, Hicks et al. 2011). Diatoms constitute the main food source for several primary consumers, which makes diatom biomass production essential for the functioning of intertidal food webs (Moens and Vincx 1997, Buffan-Dubau and Carman 2000, De Troch et al. 2012a, Moens et al. 2014, Cnudde et al. 2015). Due to their high essential fatty acid content (EFAs), diatoms are among the most critical components for the energy transfer through marine food webs (Müller-Navarra et al. 2000; Taipale et al. 2013; Guo et al. 2016, but see 1.7.2.). Moreover, benthic diatoms excrete large amounts of extracellular polymeric substances (EPS), a mix of high-molecular-weight and carbon-rich molecules (De Brouwer et al. 2000, Smith and Underwood 2000, Underwood and Paterson 2003). EPS play a beneficial role for the diatoms themselves, as a protective measure against vaious abiotic disturbances and by enabling diatom locomotion and vertical migration (Pistocchi et al. 1997, Smith and Underwood 2000, Staats et al. 2000, Gerbersdorf et al. 2009b). Moreover, EPS promote ecosystem functioning in marine coastal systems by increasing sediment stability (Decho 1990, 2000, Smith and Underwood 2000,

Gerbersdorf et al. 2009a).

Meiofauna are operationally defined as organisms which can pass a sieve with a 500 µm or 1000 µm mesh but will be retained by a 31 µm, 44 µm or 63 µm mesh, with exact dimensions varying depending on the research context (Giere 2009a). Meiofauna are among the main consumers of microalgae (Montagna et al. 1995, Buffan-Dubau and Carman 2000, Moens et al. 2002, De Troch et al. 2005) and, as potential food source for macrofauna and juvenile fish, may link microalgal primary production to higher trophic levels (Coull 1990, Aarnio and Bonsdorff 1993, McCall and Fleeger 1995, Hoyt et al. 2000, Danovaro et al. 2007, Giere 2009b). Harpacticoid copepods represent an important part of the meiofauna, and are among the main consumers (grazers) of benthic diatoms (Decho and Fleeger 1988, De Troch et al. 2005, 2012a). Their high content of EFAs, which are incorporated directly from their diatom diet, makes harpacticoids an energy-rich food source for small and juvenile fish (Nanton and Castell 1998, Wyckmans et al. 2007). As such, harpacticoid copepods represent an important link for the energy transfer from primary producers to higher trophic levels (e.g. Alheit and Scheibel 1982, De Troch et al. 1998, Buffan-Dubau and Carman 2000, Andersen et al. 2005).

Atrazine is a herbicide which binds to the plastoquinone binding protein of photosystem II, causing the disruption of photosynthetic electron flow (Dorigo and Leboulanger 2001, Legrand et al. 2006, Knauert 2008). When atrazine enters freshwater and estuarine environments through flooding events and river runoff, it can have deleterious effects on the growth and photosynthesis of aquatic primary producers, such as microalgae (Peterson et al. 1994, Bester et al. 1995, DeLorenzo et al. 2001, Pennington et al. 2001, Larras et al. 2016). Atrazine is commonly not acutely toxic to aquatic consumers, but has adverse chronic effects on consumers due to food limitation, hormonal disruption and reduced reproduction, although these reproductive and hormonal effects are not consistently observed (Jüttner et al. 1995, Bejarano and Chandler 2003, Bejarano et al. 2005, Forget-Leray et al. 2005, Solomon et al. 2008, Hayes et al. 2011). Despite its Europe-wide ban in 2004, atrazine is still a common pollutant European estuaries and remains one of the most-used pesticide worldwide (Graymore et al. 2001, Carafa et al. 2007, Noppe et al. 2007, Benbrook 2016).

In contrast to organic pesticides, heavy metals occur naturally in the environment, and several of them are essential for organism physiology (Rengel 1999, Hänsch and Mendel 2009). This is the case for copper which is involved in several metabolic pathways in microalgae, as an essential micronutrient and component of proteins and enzymes (Morelli and Scarano 2004, Hänsch and Mendel 2009). However, copper concentrations above the required levels are toxic to marine organisms at all trophic levels (Welsh et al. 1996, Millward and Grant 2000, Murray-Gulde et al.

2002, Real et al. 2003, Manimaran et al. 2012). Copper enters coastal environments through river run-off, industrial and domestic activities, copper mine drainages and agricultural practices, notably its usage in herbicides and antifouling paints (Kennish 1996, Stauber and Davies 2000, Murray-Gulde et al. 2002). Copper affects both primary producers and consumers through the formation of reactive oxygen species (ROS) which can lead to cell death by damaging cell membranes and nucleic acids (Rijstenbil et al. 1994, Knauert and Knauer 2008, Rhee et al. 2013). Copper also affects marine primary consumers by inhibiting membrane transport proteins (Bianchini et al. 2004) and by limiting the quantity of their algal diet (Pinho et al. 2007). Further copper effects on microalgae include the inhibition of electron transport in photosystem II and I (for detailed mechanisms see Miao et al. 2005, Spijkerman et al. 2007), by limiting the reduction of nitrate to nitrite (Manimaran et al. 2012) and reducing the uptake of silicon (in diatoms, Martin-Jézéquel et al. 2000).

1.8.2. Thesis outline

This work addresses four main challenges in current BEF research:

(i) contrasting BEF relations obtained with the classic design of random species loss with those induced by anthropogenic stress (addressed in Chapter 2)

(ii) identifying response and effect traits which predict the functional consequences of biodiversity loss under anthropogenic stress (addressed in Chapter 3)

(iii) determining the relative importance of the two main biodiversity effects (dominance and complementarity) for functioning in stressed communities, and identifying mechanisms which drive both effects under stress (addressed in Chapter 4)

(iv) analysing the effects of anthropogenic stress in a multitrophic context, by testing if stressors effects on producer diet quality knock on to the consumer level (addressed in Chapter 5)

Here, in the general introduction (**Chapter 1**), the scientific setting of this thesis was outlined, with a focus on the recent progress and future research scopes in BEF science. The inclusion of anthropogenic stress into BEF research was identified as first main objective, since it is unclear whether the biodiversity loss and the BEF relations obtained with the classic random BEF design correspond to those induced by anthropogenic stress. Hence, **Chapter 2** contrasts diversity gradients in diatom communities created by the classic design of random community assembly with diversity gradients induced by applying the pesticide atrazine. Next, Chapter 2 tests if BEF relations in both designs are comparable, i.e. if diversity-functioning relationships inferred from

classic random-assembly approaches predict pesticide-driven diversity-functioning relationships. Functioning is measured as the diatoms' contribution to four processes: photosynthetic efficiency and biomass production (as proxy for primary production), fatty acid production (as proxy for energy content) and EPS production (as proxy for sediment stabilization). Lastly, Chapter 2 tests if differences between the two types of diversity-functioning relationships could be explained by selective atrazine effects, i.e. to what extent the species' contribution to functioning and their stress tolerance combine in shaping BEF relations under stress.

The lacking information on the species' stress tolerance is a main reason why BEF relations are commonly tested with random species loss. **Chapter 3** therefore identifies a set of response and effect traits, which predict the abundance (numerical stress response) and functional contribution (functional stress response) of diatoms under copper and atrazine. By quantifying the effect of atrazine and copper on the abundance (cell densities) and functional contribution (photosynthetic efficiency, EPS and fatty acid production) of 17 diatom species, Chapter 3 represents a comprehensive dataset on the effects of two common chemical stressors on benthic diatoms. Moreover, by linking response and effect traits, Chapter 3 combines ecotoxicology and theoretical ecology, and provides new insight into the extent to which metal- and herbicide-tolerant species can maintain their contribution to ecosystem functioning under stress.

Dominance (dominance of communities by high- or low-yield species) and complementarity (better performances of species in community due to mechanisms such as resource partitioning or facilitation) are the two main effects which cause communities to perform differently than expected from their component monocultures (see 1.6). However, both effects have rarely been quantified under stress. Moreover, there is little ecophysiological evidence for facilitative mechanisms as driver of complementarity in stressed communities, and BEF models have rarely quantified whether complementarity differentially affects high- or low-yielding species. **Chapter 4** tests whether the biodiversity effect on diatom biomass production under copper and atrazine stress depends on dominance or complementarity effects. Moreover, Chapter 4 unravels the fundamental mechanisms driving the diversity effect on functioning, by testing if complementarity under stress is driven by the activation of facilitative mechanisms in stressed diatom communities, and by linking the degree to which species benefit from complementarity to their stress tolerance and trophic mode.

Chapter 5 relates chemical stress effects on primary producers (diatoms) to the consumer level (harpacticoid copepods). First, Chapter 5 measures the effects of atrazine and copper on the contribution of a diatom community to diet quantity (biomass production), diet quality (essential

fatty acids production) and sediment stabilization (EPS production). Next, Chapter 5 tests if diatom functioning under stress is driven by changes in community structure or by stress effects on the species' functional contribution. Last, grazer experiments are used to test if atrazine and copper effects on diatom diet quality affect the dominant grazer at the study site, the harpacticoid copepod *Microarthridion littorale*. Thereby, Chapter 5 addresses a main bottleneck issue in current BEF research, which rarely investigates stressor effects across trophic levels and nearly essentially analyzes food web functioning in terms of diet quantity but not diet quality.

The main conclusions of this work are summarized and discussed together with future perspectives in **Chapter 6**.

The **Addenda II to IV** comprise supplementary material to the chapters 2 to 5, respectively.

Addendum V reports the results of an additional experiment which quantified the tolerance of five harpacticoid species collected at the Paulina intertidal area to direct copper exposure.

Chapter 2: Stressor-induced biodiversity gradients: revisiting biodiversity – ecosystem functioning relationships

Published as:

Mensens, C., De Laender, F., Janssen, C. R., Sabbe, K & De Troch, M. (2015) Stressor-induced biodsiversity gradients: revisiting biodiversity-ecosystem functioning relationships. Oikos 124 (6): 677-684

Abstract

Biodiversity - ecosystem functioning experiments typically inspect functioning in randomly composed communities, representing broad gradients of taxonomic richness. We tested if the resulting evenness gradients and evenness-functioning relationships reflect those found in communities facing evenness loss caused by anthropogenic stressors. To this end, we exposed marine benthic diatom communities to a series of treatments with the herbicide atrazine, and analysed the relationship between the resulting gradients of evenness and ecosystem functioning (primary production, energy content and sediment stabilization). Atrazine exposure resulted in narrower evenness gradients and steeper evenness-functioning relations than produced by the design of random community assembly. The disproportionately large decrease in functioning following atrazine treatment was related to selective atrazine effects on the species that contributed most to the ecosystem functions considered. Our findings demonstrate that the sensitivity to stress and the contribution to ecosystem functioning at the species level should be both considered to understand biodiversity and ecosystem functioning under anthropogenic stress.

2.1. Introduction

Biodiversity loss due to human activities has led scientists to enquire how the diversity of communities may regulate ecosystem functioning (Cardinale et al. 2012). During the last two decades, numerous studies have shown that biodiversity favours ecosystem functioning, by positively affecting processes such as productivity or decomposition (Loreau 2000, Hooper et al. 2012). Toxic chemicals are potential drivers of biodiversity decrease (De Laender et al. 2014) but remain understudied in biodiversity-ecosystem functioning research and conservation (Lawler et al. 2006). In diversity-functioning research, ecosystem functioning is commonly measured across broad gradients of species richness in randomly assembled communities (Deutschman 2001, Díaz

et al. 2003). In the case of toxic chemicals, this makes the implicit assumption that pollution would eliminate a large number of species, and remove individuals in a random way. However, environmentally realistic levels of stressors such as chemical pollution or physical disturbance reduce the abundance of sensitive species (Davies et al. 2011), but without necessarily removing them from the ecosystem, resulting in gradients of evenness rather than species richness (Johnston and Roberts 2009). Whether the evenness gradients created by random manipulations of species richness are representative for those caused by environmental stress such as chemical toxicity remains elusive. Moreover, it is unclear how ecosystem functioning of randomly assembled communities compares to that of communities composed by environmental filtering through chemical toxicity. Chemicals can impact ecosystem functioning directly, by affecting the physiology of the concerned species (De Laender et al. 2010), and indirectly through selective biodiversity loss, depending on the species' sensitivity to the chemical (Vinebrooke et al. 2004). When species differ in their contribution to ecosystem functioning, consequences of biodiversity decline for a particular process can depend on the order in which the species are lost (Solan et al. 2004). If the most sensitive species also contributes most to a given ecosystem process, a chemical-induced reduction in density of this species, and therefore evenness reduction, can lead to more pronounced functional consequences than predicted from a random community assembly design.

We compare biodiversity gradients and diversity-functioning relations obtained using the design of random community assembly with those generated by filtering through environmental stress. First, we contrast evenness gradients created by randomly assembling 4 benthic diatom species from an intertidal mudflat with those induced by applying the herbicide atrazine, one of the most frequently used herbicides worldwide (Graymore et al. 2001). Despite its EU-wide ban, environmentally critical concentrations of atrazine are still monitored in European intertidal mudflats, which are wetlands of high economic and ecological value (Graymore et al. 2001, Niquil et al. 2006). Next, we test if changes in ecosystem functioning in both sets of experiments are comparable: Do evenness-functioning relationships inferred from the random assembly approach predict pesticide-driven non-random evenness-functioning relations? We measured three ecosystem processes and attributes that are particularly relevant in mudflat ecosystems: primary production, sediment stabilization and energy content of diatoms. Lastly, we tested if differences between the two types of diversity-functioning relationships could be explained by selective atrazine effects, by measuring to what extent species-specific contributions to ecosystem functioning and species-specific sensitivity to chemicals combine in shaping the diversity-functioning relation.

Diversity-functioning research commonly focuses on the relation between species richness and primary production (Hillebrand and Matthiessen 2009). Yet, evenness responds faster to environmental stress and can be a stronger predictor for ecosystem functioning than species richness *per se* (Chapin et al. 2000, Kirwan et al. 2007). Also, ecosystem processes other than primary production can respond differently to diversity loss (Hector and Bagchi 2007). We therefore quantified biodiversity as evenness, and analyzed its relation to three ecosystem processes and attributes. Biovolume, the most frequently used proxy for phytoplankton biomass (Hillebrand et al. 1999), and maximum quantum yield of photosynthesis, a proxy for photosynthetic efficiency (Hartig et al. 1998a, Kromkamp et al. 1998), were used to quantify primary production. Sediment stabilization was quantified as extracellular polymeric substances (EPS) production. These biopolymers secreted by microphytobenthos and other microorganisms enhance ecosystem functioning through biogenic sediment stabilization (Gerbersdorf et al. 2009a). The diatom energy content, an attribute that serves as proxy for energy transfer to higher trophic levels, was estimated by the production of polyunsaturated fatty acids (PUFAs), an essential diet component for primary consumers (De Troch et al. 2012a). PUFAs are therefore commonly used as marker for food quality and energy transfer efficiency (Brett and Müller-Navarra 1997, De Troch et al. 2012a).

2.2. Methods

Experimental organisms and culture conditions. Experimental communities were composed of 4 benthic epipelic diatom species. *Navicula arenaria, Nitzschia* sp. and *Entomoneis paludosa* were sampled from intertidal mudflats in the Westerschelde estuary (SW Netherlands, 51°21'N, 3°43'E). *Seminavis robusta* was obtained from the culture collection of the Protistology & Aquatic Ecology Research Group (UGent). Cultures of the species were maintained in a climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons $m^{-2}s^{-1}$, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients (Guillard 1975).

Atrazine and random assembly design. In the first experiment, diatom communities were constructed by random community assembly, the common approach in diversity-functioning research. All possible 15 species combinations were assembled, thereby simulating all possibilities of random species loss. In the second experiment (atrazine design), diatom communities were composed selectively through atrazine, by exposing the full species community to concentrations of 0 (control treatment), 100, 500 and 1000 µg/l atrazine respectively. Technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure) was used in

all treatments. Atrazine was dissolved in the culture medium by means of a 30 min ultrasonic bath. The resulting atrazine concentrations determined by GC-MS analysis were 91, 465 and 980 µg/l in the atrazine treatments and <1 µg/l (below detection limit) atrazine in the control treatment. Treatments in the random assembly design were run twice in polystyrene 6-well-plates, treatments in the atrazine design in 3 polysterene 6-well-plates: an equivalent of 12 and 18 replicated microcosms per treatment. Each microcosm was inoculated with a total cell density of 10 000 cells/ml (100 000 cells in 10 ml of culture medium), with equal cell densities per species. Treatments were inoculated with diatom cells from exponentially growing cultures, and incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons $m^{-2}s^{-1}$. All experiments were terminated after 18 days.

Biodiversity and ecosystem functioning measurements. Evenness was calculated every second day from cell densities per species. Cell densities were obtained by magnifying and photographing (x100) an area of 0.33 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11 digital camera). Cell numbers per species were subsequently determined by identifying and counting cells on the computer (ImageJ cell counting software) and converted into cell densities in cells/ml. Evenness for every treatment and replicate was calculated as follows:

$$J = \frac{H'}{H'_{max}}$$

$$H' = -\sum_{i=1}^{s} \frac{n_i}{N} \times \log \frac{n_i}{N}$$

Where J is eveness, H' is the Shannon index, n_i is cell density of the *i*th species. N is total cell density of the microcosm and H'_{max} is log10 of species richness (log10(4) in every treatment). Time-averaged evenness was used as biodiversity parameter in every treatment.

Biovolume was calculated every second day from cell densities and mean cell biovolume per species according to (Hillebrand et al. 1999), using measured linear dimensions and formulas representing the closest approximation of geometric shape for each genus (Addendum I Table S1). Time-averaged biovolume was calculated as proxy for primary production for every treatment.

Maximum quantum yield of photosynthetic activity was measured at 48-hour-intervals by pulse-amplitude modulated (PAM) fluorometry. Maximum quantum yield is determined as the ratio of variable and maximum fluorescence (F_v/F_m). Maximum fluorescence F_m is the maximum fluorescence emission level after a dark adaptation of 20 minutes, measured with a saturating pulse of light (emission peak at 450 nm, 2700 photons $m^{-2}s^{-1}$, 800 ms). Variable fluorescence F_v is

calculated from the difference between initial fluorescence (F_0) and maximum fluorescence ($F_v = F_m - F_0$). The data analysis used time-averaged values of maximum quantum yield for every treatment.

Extracellular polymeric substances were measured by spectrophotometry at the end of the experiments after 18 days, with 3 replicates of 10 ml suspended diatom culture per treatment. The suspended diatom cultures were centrifuged for 15 min at 15 °C and 3500*g.* The supernatant yielded the soluble EPS fraction, which was left to precipitate overnight at -20°C in 30 ml cold ethanol (98%), and subsequently centrifuged for 15 min at 15 °C and 3500*g*. The pellet was dried under a flow of nitrogen and resuspended in 2 ml of 1.5% NaCl. Samples of 200 µl of this suspension per replicate were used for the EPS analysis. EPS was measured according to a modified version of the phenol/H_2SO_4 assay by Dubois et al. (1956). In 24-well-plates, 1 ml concentrated H_2SO_4 and 200 µl phenol (5%, w / v in distilled water) were added to 200 µl sample. The mixture was shaken, incubated for 20 min and the absorbance measured at 488 nm using a Victor^3 multilabel reader (Perkin-Elmer). EPS concentrations were quantified with a function obtained from a linear regression of known concentrations of glucose standards (ranging from 0.1 to 100 µg/ml).

Fatty acid profiles in 2 replicates of 37 ml suspended diatom culture per treatment were analyzed after 18 days. The suspension was centrifuged for 20 minutes at 4 °C and 2700*g*. The supernatant was removed except 5 ml, the pellet was resuspended, transferred into a 10 ml glass tube and centrifuged for 15 minutes at 4 °C and 2700*g*. The supernatant was removed and the pellet was stored at -80 °C until fatty acid extraction. Hydrolysis of total lipid extracts of duplicate diatom cell suspensions per treatment and methylation to FA methyl esters (FAME) was achieved by a modified one-step derivatisation method (Abdulkadir and Tsuchiya 2008, De Troch et al. 2012a). The boron trifluoride-methanol reagent was replaced by 3.5 ml of a 2.5% H_2SO_4-methanol solution per sample since BF3-methanol can cause artefacts or loss of PUFA (Eder 1995). The fatty acid Methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification (see further). Samples were centrifuged and vacuum dried. The FAME thus obtained were analysed using a gaschromatograph (HP 6890N) with a mass spectrometer (HP 5973). The samples were run in splitless mode, with a 1 µL injection per run, at an injector temperature of 250°C using a HP88 column (Agilent J&W; Agilent Co., USA). The oven temperature was programmed at 50°C for 2 min, followed by a ramp at 25°C min^{-1} to 175°C and then a final ramp at 2°C min^{-1} to 230°C with a 4 min hold. The FAME were identified by comparison with the retention times and mass spectra of authentic standards and mass spectral libraries (WILEY, NITS05), and analysed with the software MSD ChemStation (Agilent Technologies). Quantification of individual FAME was accomplished by the use of external standards (Supelco # 47885, Sigma-

Aldrich Inc., USA). The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 ng/ml).

Data analysis. Biodiversity-ecosystem functioning relationships in randomly assembled and stressed communities were analyzed with a generalized least squares model (Model 1), with the respective ecosystem process or attribute as response variable and biodiversity as predictor (E[EFIa, b] = a + b·B]). B represents biodiversity, EF ecosystem functioning, and a and b the intercept and the slope (i.e. the effect of B on EF). In cases when biodiversity significantly (alpha = 0.05) predicted ecosystem functioning for both random and stressed treatments, we compared the slopes (b) and their confidence intervals between the two model fits. The absence or presence of overlap of the 95% pointwise confidence intervals for b in both models was used as a criterion for the similarity or difference of diversity-functioning relations between the atrazine-induced and random biodiversity loss. Normality and homogeneity of model residuals across the evenness range were inspected using quantile-quantile plots and by plotting residuals versus explanatory variables, respectively. If homogeneity was violated, the model was refitted using an exponential variance structure allowing residuals to change with the predictor X ($var(\varepsilon) = \sigma 2 \cdot e^{2 \cdot \delta \cdot X}$), and homogeneity was evaluated again (Model 2). By means of likelihood ratio testing, the significance of these structures was tested (alpha = 0.05).

Effects of species identity were assessed by fitting a generalized least squares model to the respective ecosystem process or attribute as response variable and the cell density of each of the 4 species as predictors [$E[EFIa, b] = a + \sum_{i=1}^{4} b_i \cdot S_i$]. EF represents ecosystem functioning, a is the intercept, S is the cell density of *Navicula arenaria, Entomoneis paludosa, Seminavis robusta* or *Nitzschia sp.* respectively, and b represents the slope, i.e. the effect of species identity on EF. Normality and homogeneity of model residuals was tested as for the diversity-functioning models, i.e. by plotting residuals vs. all predictors. All calculations were performed in R 2.10.1, using RStudio (R development Core Team 2016) and the package nlme (Pinheiro et al. 2016).

2.3. Results

Atrazine reduced diatom evenness, but to a lesser extent than random species removal. Evenness gradients obtained in the atrazine design (0.69 to 0.98) were narrower than those established in the random assembly design (0 to 0.96, Fig. 2.1). Evenness-functioning relationships differed in randomly assembled and atrazine-exposed communities. Evenness was positively related to all 4 ecosystem functioning proxies (all $p<0.0001$) in the atrazine design (Fig. 2.1, Table 2.1). In the random assembly design, evenness was significantly related to biovolume only ($p=0.0006$, Fig.

2.1, Table 2.1). Ecosystem functioning was also increasingly variable with decreasing evenness in the random assembly design (Fig. 2.1). This was confirmed by likelihood ratio testing, which showed that diversity-functioning relations in these communities were more accurately described by models including a variance structure allowing the variance of ecosystem functioning to change with evenness ($p<0.0001$; Table 2.1).

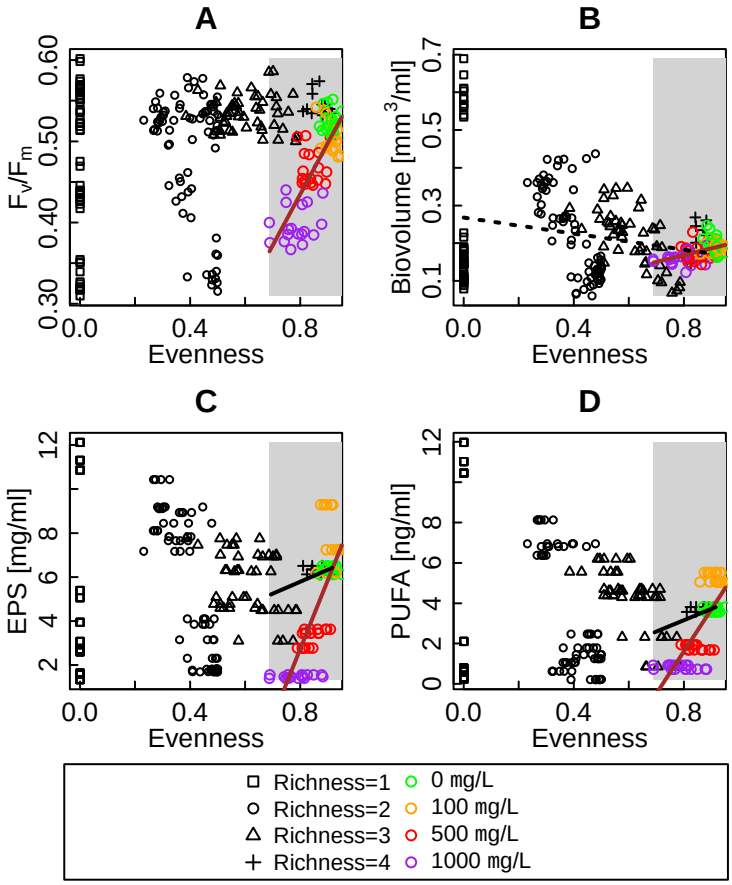


Fig. 2.1: Proxies of ecosystem functioning as a function of evenness in randomly assembled and atrazine-exposed diatom communities. Ecosystem functioning is quantified as primary production (time-averaged values of photosynthetic efficiency and biovolume, panels A and B), sediment stabilization (EPS, panel C) and energy content (PUFA, panel D). Black dots represent diatom communities from the random assembly design, coloured dots denote diatom communities from the atrazine design. The area marked in grey denotes the evenness gradient induced by atrazine. Regression lines show significant relations between evenness and functioning. Brown lines are linear models based on the atrazine design. Dashed and solid black lines are linear models based on the random assembly design for the whole evenness range or for the evenness range induced by atrazine (grey area), respectively.

To enable contrasts of evenness-functioning relations induced by the random assembly approach with those found in stressed communities within the same evenness gradient, we repeated our analyses, only considering the overlap of both evenness ranges (0.69-0.98) (Fig. 2.1, marked in grey). This was because the evenness gradient resulting from atrazine exposure was narrower

than that in the random assembly design. These analyses revealed that, in the randomly composed communities, evenness within a range of 0.69 to 0.98 was positively related to EPS (p=0.02) and PUFA (p=0.02) production (Fig. 2.1, Table 2.1). The slopes of these evenness-functioning relationships however were 3x (EPS) and 6x (PUFA) lower than those found in the atrazine design (Fig. 2.1, Table 2.1), i.e. slopes were less steep at random community composition. Diversity-functioning relationships were thus different when created by random community assembly than by exposure to atrazine.

Ecosystem Function	Data	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Primary Production: (Photosynth. Efficiency)	R	1	0.08	0.02	4.05	**0.0001**	-416	211	No		
		2	0.09	0.01	4.90	**<0.0001**	-441	224	No	*N.A.*	*N.A.*
	A	1	0.63	0.06	9.84	**<0.0001**	-264	135	Yes		
		2	0.63	0.07	8.81	**<0.0001**	-265	137	Yes	2.62	0.1057
	R‘	1	0.03	0.06	0.44	0.6619	-98	52	Yes		
		2	0.04	0.06	0.64	0.5274	-99	54	Yes	*N.A.*	*N.A.*
Primary Production: Biovolume [mm^3/ml]	R	1	-0.11	0.03	-3.01	**0.0030**	-205	106	No		
		2	-0.11	0.03	-3.50	**0.0006**	-263	136	Yes	59.82	**<.0001**
	A	1	0.18	0.04	4.56	**<0.0001**	-333	170	Yes		
		2	0.18	0.04	5.05	**<0.0001**	-332	170	Yes	0.93	0.3340
	R‘	1	0.33	0.17	1.99	0.0597	-55	30	Yes		
		2	0.42	0.16	2.59	**0.0166**	-56	32	Yes	3.05	0.0806
Sediment stabilization: EPS [mg/ml]	R	1	-0.32	0.81	-0.40	0.6895	904	-449	No		
		2	0.11	0.74	0.15	0.8835	868	-430	Yes	*N.A.*	*N.A.*
	A	1	31.12	2.91	10.69	**<0.0001**	273	-133	Yes		
		2	29.32	2.63	11.14	**<0.0001**	274	-133	Yes	1.07	0.3006
	R‘	1	6.43	3.38	1.90	0.0706	81	-38	No		
		2	5.43	2.29	2.37	**0.0272**	62	-27	Yes	21.60	**<0.0001**
Energy content: PUFA [ng/ml]	R	1	-2.36	0.91	-2.60	**0.0101**	945	-470	No		
		2	-1.05	0.73	-1.44	0.1503	874	-433	Yes	72.77	**<0.0001**
	A	1	20.83	2.09	9.94	**<0.0001**	224	-109	Yes		
		2	18.56	1.80	10.30	**<0.0001**	225	-108	Yes	1.80	0.1794
	R‘	1	4.33	3.35	1.30	0.2086	77	-36	No		
		2	6.12	2.38	2.57	**0.0175**	62	-27	Yes	17.30	**<0.0001**

Table 2.1: Relationship between evenness and ecosystem functioning in randomly composed and atrazine-exposed diatom communities. Results of generalized least squared models predicting ecosystem functioning from evenness, without (model 1) or with (model 2) a variance structure. 'Data' denotes the data on which the models are based: the classical experiment with random community assembly (R), the atrazine experiment (A), the classical experiment for evenness values overlapping with those from the atrazine experiment (R'); 's.e.' is the standard error on the estimated slopes; T and P denote the t- and p-values, bold values are statistically significant. AIC is the Akaike information criterion; 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'); 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Ecosystem Function	Data	Predictor	Slope	s.e.	T	P
Sediment stabilization: EPS [mg/ml]	A	*Navicula*	-1.31E-01	4.66E-02	-2.8243	**0.0062**
		Entomoneis	5.08E-03	2.08E-02	0.2445	0.8076
		Seminavis	9.85E-03	4.29E-02	0.2296	0.8191
		Nitzschia	2.57E-01	3.74E-02	6.8742	**<0.0001**
	R'	*Navicula*	1.94E-02	2.55E-02	0.7579	0.4578
		Entomoneis	-1.01E-02	8.76E-03	-1.1494	0.2647
		Seminavis	-4.93E-03	1.31E-03	-3.7631	**0.0013**
		Nitzschia	1.83E-02	2.27E-03	8.0803	**<.00001**
Energy content: PUFA [ng/ml]	A	*Navicula*	-1.60E+00	1.06E+00	-1.5046	0.1371
		Entomoneis	1.02E-01	4.69E-01	0.2175	0.8285
		Seminavis	-1.08E+00	9.70E-01	-1.1102	0.2709
		Nitzschia	6.12E+00	8.50E-01	7.1985	**<0.0001**
	R'	*Navicula*	2.93E-01	5.41E-01	0.5429	0.5935
		Entomoneis	-2.95E-01	1.77E-01	-1.6690	0.1115
		Seminavis	-2.12E-01	2.64E-02	-8.0345	**<0.0001**
		Nitzschia	4.89E-01	7.29E-02	6.7162	**<0.0001**
Primary Production: Biovolume [mm^3/ml]	A	*Navicula*	1.73E-01	1.28E-02	13.5532	**<0.0001**
		Entomoneis	6.97E-02	6.03E-03	11.5579	**<0.0001**
		Seminavis	-8.01E-03	1.24E-02	-0.6463	0.5203
		Nitzschia	9.59E-02	1.05E-02	9.1230	**<0.0001**
	R'	*Navicula*	1.13E-01	1.99E-02	5.6587	**<0.0001**
		Entomoneis	1.46E-01	1.56E-02	9.3537	**<0.0001**
		Seminavis	3.01E-03	2.75E-03	1.0917	0.2886
		Nitzschia	2.08E-02	1.33E-03	15.6292	**<0.0001**
Primary Production: (Photosynth. Efficidency)	A	*Navicula*	-3.41E-02	3.78E-02	-0.9010	0.3708
		Entomoneis	2.34E-02	1.96E-02	1.1984	0.2350
		Seminavis	8.30E-02	4.18E-02	1.9843	0.0513
		Nitzschia	1.23E-01	3.09E-02	3.9923	**<0.0001**
	R'	*Navicula*	-2.82E-02	3.85E-02	-0.7319	0.4732
		Entomoneis	-1.48E-04	1.89E-02	-0.0078	0.9938
		Seminavis	-4.12E-03	2.87E-03	-1.4325	0.1683
		Nitzschia	6.53E-03	2.14E-03	3.0548	**0.0065**

Table 2.2: Relationship between ecosystem functioning and diatom cell density per species in randomly composed and atrazine-exposed communities. Results of generalized least squared models predicting ecosystem functioning from cell density per species. 'Data' denotes the data on which the models are based: the atrazine experiment (A), or the classical experiment (random community assembly) for evenness values overlapping with those from the atrazine experiment (R'); 's.e.' is the standard error on the estimated slopes; bold values are statistically significant.

As we considered community performance as more relevant for ecosystem functioning than performance per cell, we initially analyzed diversity-functioning relationships in terms of absolute values. In order to know if PUFA and EPS production were also related to diversity at the cellular level, we included supplemental analyses between evenness and PUFA and EPS concentrations normalized to cell density. Normalized PUFA and evenness were not correlated in the random assembly design, but positively related in the atrazine design ($p<0.0001$, Addendum I Fig. S1, Table S2). EPS production per cell was positively correlated with evenness, both in atrazine-exposed communities and over the whole random diversity gradient (both $p<0.0001$). Similar to non-normalized values, the diversity-functioning relation was steeper when evenness gradients were induced by atrazine. In randomly assembled communities of similar evenness as induced by atrazine, the relation between evenness and normalized EPS was negative ($p=0.02$, Addendum I Fig. S1, Table S2).

In the atrazine design, diversity-functioning relations were steeper, although diversity loss was less severe than predicted by the random assembly approach. In the random assembly design, *Nitzschia sp.*, if present, always became the dominant species. Exposure to atrazine did not lead to species removal, but to a change in dominance: instead of *Nitzschia sp.*, *Navicula arenaria* dominated communities exposed to 500 and 1000 µg/l atrazine, reaching its highest cell density at the two highest atrazine concentrations. Detailed cell densities per species for every treatment are indicated in Addendum I Fig. S2 for the random assembly design and Addendum I Fig. S3 for the atrazine design. Moreover, *Nitzschia sp.* cell density was positively correlated with all proxies of ecosystem functioning, in both atrazine-exposed and randomly composed communities (Table 2.2). PUFA and EPS production, the processes that were related to evenness in both the atrazine and the random assembly design, thus also correlated with the abundance of *Nitzschia sp*. Evenness gradients found in atrazine and random assembly treatments hence coincided with a change in the identity of the dominant species (dominance of *Navicula arenaria* and *Nitzschia sp.* respectively). At high atrazine concentrations the growth of *Nitzschia sp.,* the species contributing most to all analyzed functions, was suppressed. Instead, communities were dominated by *Navicula*, which was negatively correlated with EPS production in the atrazine-exposed communities (Table 2.2).

The observed diversity-functioning relationships were thus related to the identity of the most productive species, and a selective suppression of this species by atrazine can explain the steeper decline of functioning at stressor-induced than at random biodiversity loss.

2.4. Discussion

In biodiversity-ecosystem functioning research, one typically aims at experimental biodiversity gradients that are as broad as possible, to test the effects of widespread diversity loss on ecosystem functioning (Naeem 2008). Indeed, narrow, evenness-based biodiversity gradients are uncommon in experimental diversity-functioning research (Hillebrand et al. 2008). Instead, biodiversity loss by anthropogenic activity is typically mimicked with broad diversity gradients, covering the entire range from minimal to maximal species richness of the considered ecosystem (Hillebrand et al. 2008), corresponding to evenness ranges from 0 to 1. In the case of toxic chemicals however, such extensive biodiversity loss is unlikely to happen. Realistic biodiversity loss most often only affects a fraction of the existing species pool, resulting in alterations of evenness rather than richness (Pennington et al. 2001, Hillebrand and Matthiessen 2009), even at chemical concentrations well above environmental concentrations, as represented by the highest atrazine concentrations in our study (Pennington et al. 2001). Evenness alterations induced by atrazine were less severe than those in the richness-based random assembly approach, which in this case overestimated the biodiversity loss induced by chemical stress. Yet in the random assembly design, we recorded positive effects of diversity on energy content and sediment stabilization within a narrow range of high evenness, but not over the whole broad evenness gradient. Rather than being a tradeoff to realism, we thus suggest narrow biodiversity gradients, with evenness instead of richness as diversity proxy, as a more realistic simulation of pollution-induced biodiversity loss that can facilitate the detection of biodiversity effects on ecosystem functioning.

Diversity-functioning relations were steeper when diversity gradients were chemically-induced rather than randomly assembled, denoting a disproportionately large decrease of functioning in diatom communities under stress conditions. Contrasting the design of random community assembly with scenarios of selective biodiversity loss due to environmental filtering is among the main challenges for future diversity-functioning research (Srivastava 2002, Giller et al. 2004), but has rarely been tested experimentally (Symstad and Tilman 2001, Smith and Knapp 2003, Solan et al. 2004, Larsen et al. 2005). Chemical stress moreover has not been studied as driver for realistic biodiversity loss and ecosystem functioning, although toxic chemicals emitted by industry, agriculture and households are among the most potent threats to aquatic species (Geiger et al. 2010). We found a larger discrepancy in random and stressor-induced diversity-functioning relations (3 to 6 time steeper relations in stressed communities in terms of EPS and PUFA production) than in other cases where the random assembly design could be contrasted with realistic diversity loss (Smith and Knapp 2003, Solan et al. 2004, Schläpfer et al. 2005, Larsen et al. 2005). This can be due to the organisms and ecosystem processes studied, and to selective atrazine effects on the physiological rates and composition of the diatom communities.

In our diatom communities that were randomly assembled, primary production, the most common measure of ecosystem functioning (Hooper et al. 2005), was not related to diversity. In contrast, sediment stabilization and energy content showed a steeper relation to diversity than primary production in both the atrazine and the random assembly design, which facilitated the contrast of the two experimental approaches. The observed functional consequences of stressor-induced diversity loss thus depended on the measured ecosystem function, which underlines the value of analyzing multiple ecosystem processes (Hector and Bagchi 2007, Hiddink et al. 2009). Also, the pool of 4 species could have contributed to the steep diversity-functioning relations. In our experimental communities, the functionally most important species was most affected by atrazine (but see below). In a larger species pool, this selection effect could be compensated by functionally redundant, but less sensitive species. Moreover, as randomized and stress-induced biodiversity and ecosystem functioning have so far not been assessed in marine microalgae, we cannot relate our findings to other studies using similar organisms. The disproportionate loss of functioning following selective loss of diatom biodiversity however corresponds to existing knowledge on non-random diversity loss in grassland primary producers (Symstad and Tilman 2001, Zaveleta and Hulvey 2004). Yet, the steep diversity-functioning relationships in our stressed communities can not only be explained by the number and type of different organisms and ecosystem processes. Loss of functioning in stressed communities can be related to selective alterations of biodiversity by the stressor, as well as direct stressor effects (Schimel et al. 2007). In our case, atrazine could affect functioning indirectly through selective changes of community composition and directly by reducing physiological rates of the species. Atrazine treatments in which functioning was reduced with regard to the control were essentially dominated by *Navicula arenaria*, the only species which was able to grow at the highest atrazine concentration. To inspect potential direct physiological effects, we compared PUFA and EPS production per cell in these *N. arenaria*-dominated stressed communities with those of unexposed *N. arenaria* monocultures. Cells in the *N. arenaria*-dominated communities produced 1.8 and 3.5 times less EPS, but 2 and 3 times more PUFA than in the monocultures of this species (Addendum I Fig. S4). The reduction in EPS in stressed communities could thus be due to physiological effects of atrazine on *N. arenaria*. The reduction in PUFA however does not seem to be caused by physiological effects, as *N. arenaria* actually seems to increase its PUFA production when exposed to atrazine. To understand the steep loss of functioning in our stress-exposed communities, we must therefore, depending on the ecosystem process, look beyond direct stressor effects and consider selective atrazine effects on the community composition.

In diversity-functioning research, consequences of biodiversity loss are commonly predicted using randomly assembled communities, whereas environmental filtering due to stress results in distinct non-random community compositions. Furthermore, evenness is often held constantly

high across species richness treatments (Wilsey and Polley 2004), thus minimizing effects of relative abundances (i.e. species identity) on ecosystem functioning. Due to the increasing recognition that biodiversity and species identity combine in shaping ecosystem functioning (Bruno et al. 2006), research on realistic biodiversity loss has started by removing rare and uncommon species from the community (Smith and Knapp 2003). Despite making the step from random to a more realistic, abundance-based biodiversity loss, this approach still assumes that the naturally abundant species are also most resistant to environmental stressors and less likely to be lost. In our diatom communities, *Nitzschia sp.* was the most abundant species in the natural samples as well as in the experimental communities, contributing most to the measured ecosystem processes. However, when exposed to stress, *Nitzschia sp.* was the species whose cell density was most reduced by atrazine. This shows that both in stressed and randomly assembled communities, community composition tends to be uneven, and that the most abundant species is not necessarily the most stress-resistant. Biodiversity loss generally reduces ecosystem functioning, but general physiological and selective stressor effects on the most abundant and functionally most efficient species lead to a disproportionate loss of ecosystem functioning in stressed communities compared the random assembly design.

Benthic diatoms are the dominant primary producers and food source in many intertidal, soft-sediment environments as well as on rocky shores (Tang and Kristensen 2007, Speybroeck et al. 2008). Diatom communities play a pivotal role in controlling energy flow, nutrient fluxes and productivity in these systems (Underwood and Kromkamp 1999, De Troch et al. 2012a). Diatom biodiversity loss resulting in a two-fold reduction in diatom biomass, three-fold reduction of unsaturated fatty acids and six-fold decrease of EPS production would strongly impact the ecosystem under concern. The standing stock and energy flux to higher trophic levels would be affected (Decho 1990, Müller-Navarra et al. 2000), zoobenthos and as a consequence fish biomass production limited (De Troch et al. 2012a), and sediment erosion promoted. Concomitantly, knock-on effects on the variety of processes undertaken by sediment-associated organisms collectively contributing to ecosystem functioning can be expected (Tolhurst et al. 2003). Stressor-induced biodiversity loss at the base of the marine food web could thus affect ecosystem functioning more seriously than predicted by the random assembly design in diversity-functioning research. In our study, the random assembly approach overestimated biodiversity loss, but underestimated the associated loss of ecosystem functioning. These contrasting results strengthen the need to more realistically predict biodiversity gradients and diversity-functioning relations caused by anthropogenic stress, by considering the prevailing stressors, and the sensitivity and contribution to ecosystem functioning of the concerned species.

In conclusion, we infer three general statements from our results. First, existing diversity-functioning research largely overestimates the biodiversity gradients induced by common environmental stressors such as herbicides. Second, diversity-functioning relations differ depending on whether the diversity gradient is generated by random community assembly, or by stressor-induced non-random assembly. Third, physiological stressor effects can affect ecosystem functioning, and the slope of the diversity-functioning relation depends on stressor-induced dominance shifts.

Acknowledgements

CM acknowledges the Doctoral Programme on Marine Ecosystem Health and Conservation (MARES) for his doctoral research fellow grant. The authors acknowledge financial support from the research council of Ghent University (project BOF-GOA 01GA1911W) for the lab supplies. MDT is a postdoctoral researcher financed by the latter project. FDL acknowledges the Research Foundation Flanders (FWO) for his postdoctoral research fellow grant. The authors thank the anonymous reviewers for their constructive remarks that contributed to the improvement of the manuscript.

Chapter 3: Different response-effect trait relationships underlie contrasting numerical and functional responses to two chemical stressors

Under revision:

Mensens, C., De Laender, F., Janssen, C. R., Sabbe, K & De Troch, M. (2016) Different response-effect trait relationships underlie contrasting numerical and functional responses to two chemical stressors. Journal of Ecology

Abstract

Trait-based approaches predict ecosystem functioning under environmental change by relating response traits (predicting changes in species' densities) to effect traits (determining the contribution to functioning). Stressors can however drive ecosystem functioning not only by altering species densities, but also by directly changing species' effect traits.

We first identified the response traits predicting the cell density of 18 marine benthic diatom strains along gradients of two chemical stressors (a pesticide and a metal, atrazine and copper). We then tested if response traits could predict stressor-induced changes in the effect traits driving the diatom's potential contribution to primary production, sediment stabilization and energy content in intertidal systems. Finally, we examined if changes in density and in effect traits were correlated, i.e. whether species able to grow under stressful conditions could maintain their contribution to ecosystem functioning.

The relationship between response traits and stressor-induced changes in density and effect traits was different depending on stressor type: a set of intercorrelated morphological traits predicted changes in both density and effect traits under metal stress, with large cells being more stress-resistant. Metal effects on density and on effect traits were positively related: diatoms whose density was least affected by the metal were also able to sustain functioning under metal exposure.

In contrast, the capacity for mixotrophic growth predicted changes in density, but not changes in effect traits under pesticide stress. Pesticide effects on density and on effect traits were negatively related for energy content and sediment stabilization, indicating a limited capacity of pesticide-tolerant diatoms to maintain their contribution to ecosystem functioning.

Ecosystem functioning under stress can depend on whether response traits driving effects on density also predict changes in effect traits. Based on our results, we expect a disproportionate

loss of functioning when traits driving species densities do not allow to maintain the contribution to functioning under stress.

3.1. Introduction

Understanding and predicting ecosystem functioning under environmental change has become a focus in ecological research due to the impact of human activities on natural ecosystems and the goods and services they deliver (Cardinale et al. 2012; Hautier et al. 2015; De Laender et al. 2016). Environmental change can affect ecosystem functioning by causing species loss (Cardinale et al. 2012; Naeem et al. 2012; Tilman et al. 2014), but also by altering species densities and their per-capita contribution to ecosystem functioning (Fox 2006; De Laender et al. 2016).

Trait-based frameworks are available to evaluate ecosystem functioning under environmental change, by relating traits predicting species densities (response traits) to traits driving the contribution to functioning (effect traits, *sensu* Suding et al. 2008; Hillebrand and Matthiessen 2009). Response-effect-trait frameworks have proven powerful predictors of ecosystem functioning, since response traits often correspond to effect traits (see e.g. Lavorel and Garnier 2002; Larsen et al. 2005; Pakeman 2011; Eklöf et al. 2012; Díaz et al. 2013; Karp et al. 2013; Heuner et al. 2015). Conversely, the insurance effect (Naeem and Li 1997) is at work if response traits are uncorrelated to effect traits, which buffers ecosystem function under environmental change (Lavorel and Garnier 2002; Eklöf et al. 2012). Empirical tests of the response-effect trait framework are however rare (Klumpp and Soussana 2009) since current knowledge on traits for biota other than terrestrial plants remains limited (Lavorel et al. 2013), which restrains our capacity to predict ecosystem functioning for different types of communities.

Toxic chemicals are common abiotic stressors, which may cause widespread changes in species densities (Malaj et al. 2014). Chemical-induced changes in species densities have been linked to response traits (Liess and Von Der Ohe 2005; Baird and Van den Brink 2007; Beketov et al. 2009; Larras et al. 2012). However, it is far from known if effect traits are themselves unaffected by chemical stress. Stressors which alter species densities are also likely to directly change the organisms' contribution to functioning (Relyea and Hoverman 2006; Schimel et al. 2007; De Laender et al. 2010). This is at least the case for chemical stressors, which are known to alter traits that are relevant to ecosystem functioning (Relyea and Hoverman 2006; Rohr et al. 2006).

Changes in species densities (hereafter numerical stress response) and changes in effect traits (hereafter functional stress response) have not yet been linked, but are essential for the application of trait-based frameworks to predict ecosystem functioning under stress. If the

numerical and functional stress response are positively related, a stressed community could still be expected to retain most of its functioning if numerically tolerant species are not affected in their effect traits (Fig. 3.1a). If the numerical and functional response are uncorrelated, the capacity to maintain functioning under stress is not related to changes in density (Fig. 3.1b). Alternatively, stress exposure could lead to a disproportionate reduction in functioning if numerically tolerant species, while still being able to grow, would lose most of their contribution to functioning (Fig. 3.1c). Stressor effects on ecosystem functioning would then be more important than merely estimated from species densities.

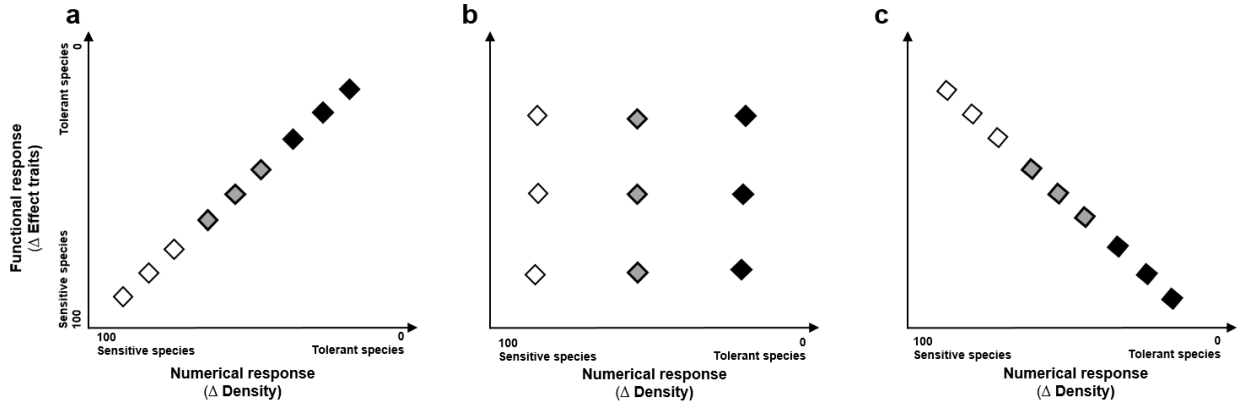


Fig. 3.1: Possible relationships between the numerical stress response (changes in density, predicted by response traits) and the functional stress response (changes in effect traits). Black, grey and white symbols represent species with respectively high, intermediate and low stress tolerance in terms of density. Numerical and functional response are indicated on a scale of 0 to 100, ranging from no (0) to maximal (100) changes in density or in effect traits.

If the numerical and functional response are positively related (a), stress changes the effect traits of species which cannot maintain their density under stress (white symbols). Species capable of maintaining their density under stressful conditions (black symbols) experience little changes in their effect traits and might be able to maintain ecosystem functioning.

If the numerical and functional response are uncorrelated (b), changes in density are independent of any concomitant change in effect traits, and numerically tolerant and sensitive species are similarly effected in their contribution to ecosystem functioning.

If the numerical and functional response are negatively related (c), the species maintaining their effect traits under stress are most affected in their density (white symbols), whilst species reaching high densities under stressful conditions are most affected in their effect traits (black symbols), resulting in a disproportionate loss of functioning under stress.

Figure modified from: Suding et al. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology 14:1125–1140.

In the present paper, we relate the traits driving the numerical and functional response of 18 benthic diatom strains to metal (copper) and pesticide (atrazine) stress. Benthic intertidal diatoms play a pivotal role in the functioning of estuarine ecosystems, where they can provide up to 50% of primary production (Underwood and Kromkamp 1999), enhance sediment stabilization through the secretion of extracellular polymers (Decho 2000), and are of essential importance for trophic energy transfer (Dunstan et al. 1993; Müller-Navarra et al. 2000). Copper is a common pollutant in coastal ecosystems due to urban and industrial runoff and its use in herbicides and antifouling paints (Kennish 1996; Murray-Gulde et al. 2002), and atrazine represents one of the most-used agricultural herbicides worldwide (Graymore et al. 2001; Chirnside et al. 2007).

We test three research questions (Fig. 3.2). First, we examine whether the numerical stress response in diatoms can be predicted by a set of seven response traits (biovolume, surface-to-volume ratio, cell length, total organic carbon, total nitrogen, Chlorophyll *a* content and mixotrophy), which have been linked to algal responses to environmental change (Debenest et al. 2009; Finkel et al. 2010; Kruk et al. 2010; Larras et al. 2014).

Next, we analyze if the above traits can predict the diatoms' functional stress response (changes in the effect traits driving the contribution to primary production, sediment stabilization and energy content), i.e. whether changes in functioning can be predicted from the same traits as changes in density.

Last, we test if and how the numerical and functional response are related, for example if species that are tolerant in terms of density (i.e. their growth is unaffected) are also able to maintain their contribution to ecosystem functioning under chemical stress.

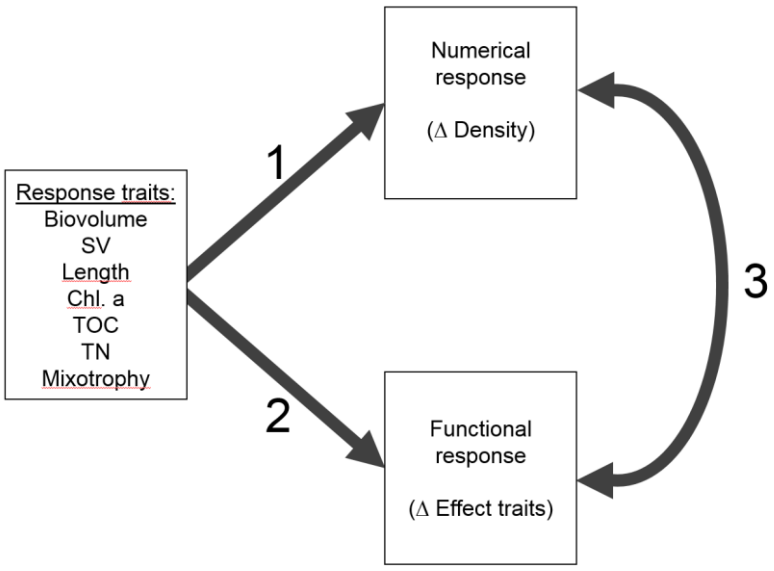


Fig. 3.2: Scheme of the three research questions investigated: Question 1 asks if response traits can predict the numerical stress response of diatoms (changes in cell density) to copper and atrazine. Response traits are biovolume, surface-to-volume ratio (SV), cell length, Chlorophyll a content (Chl. a), total organic carbon (TOC), total nitrogen content (TN) and the capacity for mixotrophic growth. Question 2 asks if response traits can predict the functional stress response (changes in the effect traits driving the diatoms' potential contribution to primary production, sediment stabilization and energy content). Question 3 asks if the numerical and functional stress response are related, i.e. if diatoms which are little affected in their density can maintain their contribution to ecosystem functioning and vice versa.

3.2. Methods

Experimental organisms and culture conditions. In total 18 diatom strains (belonging to 17 species, Addendum II Table S1) were isolated from intertidal mudflats in the Westerschelde estuary (The Netherlands, 51°21'N, 3°43'E) and the Veerse meer (The Netherlands, 51°32'N, 3°47'E), where diatoms represent the dominant component of the microphytobenthos (Forster et al. 2006). The strains were deposited in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be); accession numbers are indicated in Addendum II Table S1. Diatoms were maintained in xenic cultures in a climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons $m^{-2}s^{-1}$, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients (Guillard 1975).

Experimental setup. We performed three experiments (see experimental setup in Addendum II Fig. S2). In experiment 1 and 2, diatoms were exposed to concentrations of 0, 50, 100, 200, 500 and 1000 µg/L atrazine and 0, 10, 30, 90, 270 and 810 µg/L copper, respectively. Concentrations were based on trial tests (data not shown) as well as published sensitivity data for diatoms (Stauber and Davies 2000; Pérez et al. 2006; Wood et al. 2014) and were prepared from atrazine and copper stock solutions. The atrazine stock solution was prepared by dissolving 50 mg technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure, Sigma-Aldrich Chemie Gmbh, Munich, Germany) in 10 mL acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.025% acetone in the treatments. An acetone control treatment of 0.025% acetone was included and compared to an acetone-free control to test for carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a $Cu[II]Cl_2$ solution, analytical grade; VWR International) was spiked directly into the culture medium before exposure of the diatoms. F/2 culture medium for the copper experiments was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The resulting atrazine and copper concentrations are indicated in Addendum II Table S3. In

experiment 3, each diatom strain was grown in 4 treatments: (a) f/2 culture medium, (b) f/2 medium + atrazine (at EC50 concentration), (c) f/2 medium + atrazine + 0.1 mM Glucose (d) f/2 medium + atrazine + 0.6 mM Glucose. The obtained cell densities were used to calculate each strain's capacity for mixotrophic growth (see below). The glucose concentrations of experiment 3 were set as described in the mixotrophy experiment of Lewin and Hellebust (1975). All three experiments were run in polysterene 6-well-plates, an equivalent of 6 replicated microcosms per treatment. Each microcosm was inoculated with a total density of 5000 diatom cells/mL from exponentially growing cultures (50 000 cells in 10 mL of culture medium). Microcosms were incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons $m^{-2}s^{-1}$. Culture medium was refreshed after 12 days, and all experiments were terminated after 25 days.

Cell densities. Cell densities were obtained in six replicates per treatment on days 0, 2, 5, 10, 15, 20 and 25 of all three experiments by magnifying and photographing (x100) an area of 0.33 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11 digital camera). Cell densities (in cells/mL) were subsequently determined by digitally counting the cells by means of ImageJ cell counting software (Schneider et al. 2012).

Response traits. Morphological traits (biovolume, cell length, surface-to-volume ratio) and Chlorophyll *a* content were measured in experiment 1 and 2 in six replicates per treatment. Morphological traits were obtained from microscopic images (see above). The linear dimensions of diatom cells were digitally measured using ImageJ software calibrated to a stage micrometer and a standardized set of equations representing the closest approximation of geometric shape for each genus (Hillebrand *et al.* 1999). Chlorophyll *a* content was measured *in vivo* on days 0, 2, 5, 10, 15, 20 and 25 by PAM fluorometry (MAXI Imaging PAM fluorometer, Walz, Germany) as the minimum chlorophyll fluorescence signal (F_0) after 15 minutes of dark-adaptation.

Total organic carbon (TOC), total nitrogen (TN) and mixotrophy were measured in experiment 3. TOC and TN were measured in the control treatment after 25 days in two replicates of 10 mL suspended diatom culture. Diatom suspensions were centrifuged 10 minutes at 4 °C and 1000*g*. The pellets were freeze-dried, homogenized and acidified with 1% HCl and TOC and TN content were measured using a Flash EA 1112+ MAs 200 elemental analyser (Thermo Interscience). Mixotrophy was measured as the capacity to compensate growth inhibition by atrazine in the presence of organic carbon sources, and was calculated as the ratio of the maximum cell density that each strain reached under atrazine and atrazine + 0.1 mM and 0.6 mM glucose, respectively (see also experimental setup in Addendum II Fig. S2).

Effect traits. Effect traits were measured in all treatments of experiment 1 and 2 as the contribution per diatom cell to three processes that are crucial for ecosystem functioning in intertidal systems. Maximum light use efficiency in photosystem II (hereafter photosynthetic efficiency), an essential component in primary production, was measured as maximum quantum yield of photosynthesis in photosystem II (Cleveland et al. 1989; Consalvey et al. 2005). The potential contribution to sediment stabilization was estimated as extracellular polymeric substances (EPS) production. These biopolymers secreted by microphytobenthos enhance ecosystem functioning through biogenic sediment stabilization (Decho 2000). The diatom energy content for higher trophic levels was quantified as the production of polyunsaturated fatty acids, an essential diet component for primary consumers and a marker for energy transfer efficiency (Brett and Müller-Navarra 1997; De Troch et al. 2012).

Photosynthetic efficiency was measured as maximum quantum yield of photosynthetic activity in six replicates per treatment on days 0, 2, 5, 10, 15, 20 and 25 by pulse-amplitude modulated (PAM) fluorometry (MAXI Imaging PAM fluorometer, Walz, Germany). Maximum quantum yield is determined as the ratio of variable and maximum fluorescence (F_v/F_m). Maximum fluorescence F_m is the maximum fluorescence emission level after a dark adaptation of 20 minutes, measured with a saturating pulse of light (emission peak at 450 nm, 2700 photons $m^{-2}s^{-1}$, 800 ms). Variable fluorescence F_v is calculated from the difference between initial fluorescence (F_0) and maximum fluorescence ($F_v = F_m - F_0$).

EPS were measured by spectrophotometry at the end of the experiments after 25 days. Three replicates of 10 mL suspended diatom culture per treatment were centrifuged for 15 min at 15 °C and 3500*g.* The supernatant yielded the soluble EPS fraction, which was left to precipitate overnight in 30 mL cold ethanol (98%), and subsequently centrifuged for 15 min at 15 °C and 3500*g*. The pellet was dried under a flow of nitrogen and resuspended in 2 mL of 1.5% NaCl. Samples of 200 µL of this suspension per replicate were used for the EPS analysis. EPS was measured according to a modified version of the phenol/H_2SO_4 assay (Dubois et al. 1956). In 24-well-plates, 1 mL concentrated H_2SO_4 and 200 µl phenol (5%, w / v in distilled water) were added to 200 µL sample. The mixture was shaken, incubated for 20 min and the absorbance measured at 488 nm using a Victor³ multilabel reader (Perkin-Elmer). EPS concentrations were quantified with a function obtained from a linear regression of known concentrations of glucose standards (ranging from 5 to 400 µg/mL).

Fatty acid profiles in two replicates per treatment were analyzed after 25 days. A suspension of 10 mL suspended diatom culture was centrifuged for 20 minutes at 4 °C and 2700*g*. The supernatant was removed except for 1 mL, the pellet was resuspended, placed in a glass vial and

stored at -80 °C for fatty acid analysis. Hydrolysis of total lipid extracts of diatom cell suspensions and methylation to FA methyl esters (FAME) was achieved by a modified one-step derivatisation method (Abdulkadir and Tsuchiya 2008; De Troch et al. 2012). The boron trifluoride-methanol reagent was replaced by 3.5 mL of a 2.5% H_2SO_4-methanol solution per sample since BF_3-methanol can cause artefacts or loss of fatty acids (Eder 1995). The fatty acid methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification (see further). Samples were centrifuged and vacuum dried. The FAME thus obtained were analysed using a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973). The samples were run in splitless mode, with a 1 µL injection per run, at an injector temperature of 250°C using a HP88 column (Agilent J&W; Agilent Co., USA). The oven temperature was programmed at 50°C for 2 min, followed by a ramp at 25°C min^{-1} to 175°C and then a final ramp at 2°C min^{-1} to 230°C with a 4 min hold. The FAME were identified by comparison with the retention times and mass spectra of authentic standards and mass spectral libraries, and analysed with the software MSD ChemStation (Agilent Technologies). The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the external standards (Supelco # 47885, Sigma-Aldrich Inc., USA) ranging from 5 to 250 ng/mL).

All response and effect traits were normalized to cell density and are indicated in Addendum II Table S1.

Data analysis

The numerical stress response was quantified as EC50, i.e. the effective copper and atrazine concentration inducing a 50% loss of each species' maximum cell density. The functional stress response was quantified as the EC50 of photosynthetic efficiency, EPS and fatty acids production. EC50 values as a sensitivity endpoint represent the baseline for pesticide risk assessment in Europe (Van den Brink et al. 2011) and were calculated using a three-parameter log-logistic model (Equation 1) and a three-parameter Weibull model in two different parameterisations (Equations 2 and 3) with the R package drc as described in Ritz and Streibig (2005).

$$Y = d / (1 + \exp\{b[\log x - \log e]\}) \quad [1]$$

$$Y = d(1 - \exp\{ - \exp[b(\log x - \log e)]\}) \quad [2]$$

$$Y = d(\exp\{ - \exp[b(\log x - e)]\}) \quad [3]$$

Y represents the response variable (cell density, photosynthetic efficiency, EPS or fatty acids per cell), b the relative slope of the curve, d the upper limit, e the inflection point, and x the concentration of atrazine or copper.

To account for the hormetic growth and EPS production of several species under stress (i.e. higher cell densities and EPS production at low atrazine and copper concentrations than under control conditions), dose-response models that can describe hormesis were fitted as described by Brain and Cousens (1989, Equation 4) and Cedergreen et al. (2005, Equation 5).

$$Y = c + (d - c + fx)/(1 + \exp\{b[\log x - \log e]\}) \quad [4]$$

$$Y = (d + f \exp(-1 / x^{\alpha})) / (1 + \exp(b(\log x - \log e))) \quad [5]$$

c is the lower and *b* the upper horizontal asymptote and *f* is the hormesis effect. Model 5 was fitted with α = 1, 0.5 and 0.25, respectively. Model fit for every dose-response model was evaluated visually as well as by likelihood ratio testing, lack-of-fit test and Akaike information criterion (AIC), and the best-fitting model was used to calculate EC50 values.

The relations between response traits and the numerical and functional response (research questions 1 and 2) respectively were analyzed with generalized least squares (GLS) models (Equation 6), with numerical and functional response as response variable and traits as predictors (biovolume, surface-to-volume ratio, length, Cholorophyll a content, mixotrophy at 0.1 and 0.6 mM Glucose, TN and TOC). Since we measured three traits (mixotrophy, TOC and TN) in different experiments than the numerical and functional response to atrazine and copper (Addendum II Fig. S2), all models in Equation 6 were not fitted to the replicates as independent measurements but to the mean EC50 and trait values.

$$R = a + \sum(b_i \cdot T_i) \quad [6]$$

T_i represents the traits, R the numerical and functional response (EC50) in terms of respectively cell density, and photosynthetic efficiency, EPS and fatty acid production, a the intercept and b_i the slope (i.e. the effect of trait i on stress response). GLS models were fitted to all uncorrelated traits (correlation factor < 0.5, Addendum II Table S4) as predictors, correlated traits were separately included as predictors in additional model fits. Models were selected with a backward stepwise elimination of predictors, with model section based on AICc. At each step, the predictor whose removal resulted in the best model fit (lowest AICc) was eliminated. This process was repeated until a further removal of predictors did not improve the model fit. When a trait significantly predicted the numerical or functional stress response, we tested in additional analyses if the respective trait also predicted stress response when species with outlier trait values were omitted.

The relation of numerical and functional stress response (research question 3) was assessed by fitting GLS models to the numerical response as predictor and the respective functional response as response variable (Equation 7).

$$R_F = a + b \cdot R_D \quad [7]$$

R_F is the functional stress response in terms of photosynthetic efficiency, EPS and fatty acid production respectively, a is the intercept, R_D the numerical response in terms of cell density, b represents the slope, i.e. the relation of numerical and functional response.

For all GLS models, normality and homogeneity of model residuals were evaluated using quantile-quantile plots and by plotting residuals versus explanatory variables, respectively. If homogeneity was violated, the model was refitted using an exponential variance structure (R package nlme, Pinheiro et al. 2016) allowing residuals to change separately with each predictor X ($var(\varepsilon) = \sigma 2 \cdot e^{2 \cdot \delta \cdot X}$), the best-fitting model (lowest AIC) was selected, and homogeneity was tested again. By means of likelihood ratio testing, the significance of this structure was tested (alpha = 0.05). Pseudo-R2 values were calculated from the log-likelihoods of the fitted GLS models using the r.squaredLR function in the R package MuMIn (Barton 2016). All calculations were performed in R 3.0.1, using RStudio (R Development Core Team 2016).

3.3. Results

Changes in diatom cell density were related to cell biovolume, with larger species being more tolerant to copper (Fig. 3.3a, Addendum II Table S5). Other morphological traits that were correlated with biovolume (surface-to-volume ratio, length) also predicted cell density under copper in separate model fits (Addendum II Table S6). In contrast, diatom cell density under atrazine was not related to any morphological trait, but to the capacity for mixotrophic growth (Fig. 3.3b, Addendum II Table S5). Diatom species capable of mixotrophic growth turned out to be least affected by atrazine (see mixotrophic growth response and atrazine sensitivity per species in Addendum II Table S1 and S8). Biovolume and mixotrophy also predicted copper- and atrazine-induced changes in cell density in additional analyses that omitted the largest and most mixotrophic species, respectively (*Entomoneis* sp. and *Cylindrotheca closterium*, data points in upper right quadrant of Fig. 3.3a and 3.3b, Addendum II Table S6).

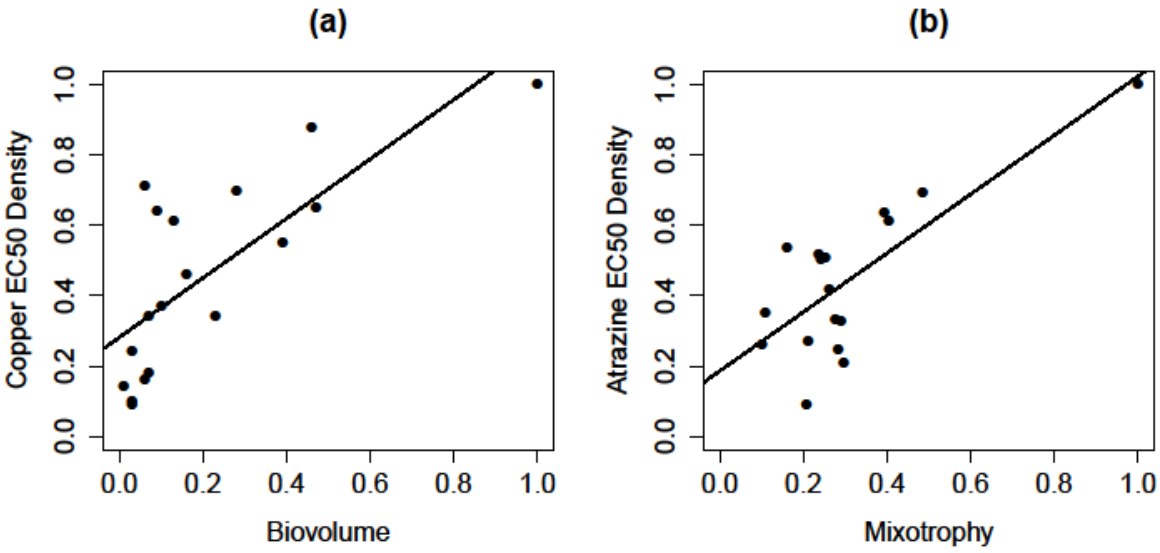


Fig. 3.3: Copper (panel a) and atrazine (panel b) EC50 on diatom cell density (numerical response) as a function of biovolume and mixotrophy, respectively. Mean EC50, biovolume and mixotrophy values are normalized to the highest respective value recorded, lines indicate significant regressions.

Changes in all three effect traits under copper were predicted by biovolume, with larger species being least affected in their photosynthetic efficiency, fatty acid content and EPS production (Fig. 3.4a, Addendum II Table S5). Biovolume also predicted changes in fatty acids and EPS, but not photosynthetic efficiency, when the largest species (*Entomoneis* sp.) was omitted from the analysis (Addendum II Table S6). In additional model fits where biovolume was replaced by correlated traits (cell length and surface-to-volume ratio respectively) changes in EPS were also predicted by cell length and changes in fatty acid content were predicted by cell length and surface-to-volume ratio (Addendum II Table S6). In the presence of atrazine, changes in photosynthetic efficiency, fatty acid and EPS production were not correlated to any of the response traits (Fig. 3.4b, Addendum II Table S5), if outlier data were omitted from the analysis (Addendum II Table S6). EC50s for all diatom strains are indicated in Addendum II Table S8, and the growth curves for every strain and stressor are shown in Addendum II Fig. S10. The diatoms' morphological traits were not altered during exposure to atrazine and copper, with the exception of *Astartiella bahusiensis*, which increased its cell size when exposed to copper (Addendum II Fig. S9, Table S9).

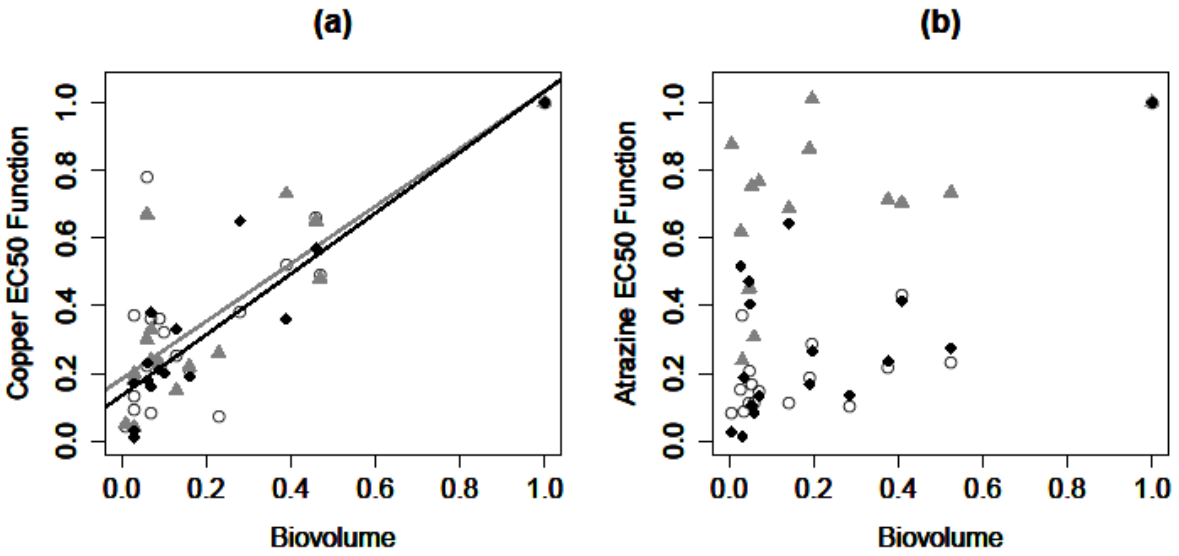


Fig. 3.4: Copper (panel a) and atrazine (panel b) EC50 on diatom effect traits (functional response) as a function of biovolume. Mean EC50 and biovolume of each diatom strain are normalized to the highest respective value recorded, lines indicate significant regressions. Effect traits are photosynthetic efficiency (white circles), EPS (grey triangles, grey line) and fatty acid production (black diamonds, black line).

Changes in cell density and changes in effect traits were related. However, this relationship differed between copper and atrazine. For copper, species which were most tolerant in terms of cell density were also least affected in their effect traits. Changes in density and all three effect traits were positively related (Fig. 3.5a, Addendum II Table S7). A similar positive relation was observed between the changes in cell density and changes in photosynthetic efficiency under atrazine (Fig. 3.5b, Addendum II Table S7). Conversely, atrazine-induced changes in density and changes in effect traits in terms of EPS and fatty acid production were negatively related (Fig. 3.5b, Addendum II Table S7). These negative relations between density changes and effect trait changes under atrazine were largely driven by species whose density was least affected by atrazine (e.g. *Cylindrotheca closterium*, *Gyrosigma* sp. 2, *Cylindrotheca fusiformis*, *Biremis ambigua*), but which were amongst the most atrazine-sensitive ones in terms of their contribution to functioning (Addendum II Table S8). Changes in effect traits were thus not only related to response traits, but also to the species' capacity to maintain their density under stress: the changes in density and changes in effect traits under atrazine were not driven by the same set of response traits, and atrazine-resistant species in terms of density were amongst the most sensitive in their EPS and fatty acid production. In the presence of copper, changes in effect traits and changes in density were driven by the same morphological traits, and density-tolerant species were also least affected in their contribution to functioning.

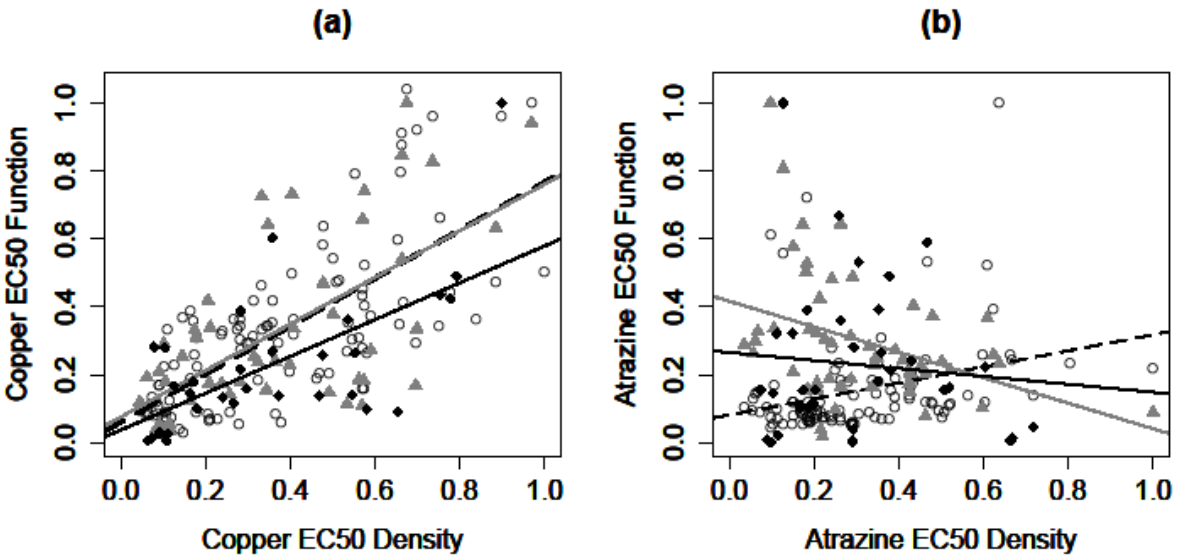


Fig. 3.5: Copper (panel a) and atrazine (panel b) EC50 on diatom effect traits (functional response) as a function of copper and atrazine EC50 on diatom cell density (numerical response). Significant relations are indicated by regression lines. Effect traits are photosynthetic efficiency (white circles, dashed line), EPS (grey triangles, grey line) and fatty acid production (black diamonds, black line). Depicted EC50 values of each diatom strain are the six (photosynthetic efficiency), three (EPS) and two (fatty acids) independent replicates. All EC50 values are indicated normalized to the highest respective value recorded.

3.4. Discussion

The outcome of our experiments demonstrates that the numerical and functional response of benthic diatoms to two common chemical stressors can partly be predicted from traits, but also that these stressors are likely to have a differential impact on ecosystem functioning as the relationship between numerical and functional response is stressor specific. Response traits predicted both the numerical and functional response to copper and the numerically tolerant species were least affected in their functioning, suggesting that the first appearance of metal effects on density will probably have little effect on overall ecosystem functioning. In contrast, mixotrophic growth can help to maintain the density but not the functioning of certain species in response to photoinhibiting stressors such as atrazine, pointing at a limited capacity of numerically tolerant species to maintain ecosystem functioning.

Morphological traits appear as promising tools to predict the numerical and functional response of algae to environmental change, since they are relatively simple to measure, capture most of the functional properties of microalgae, and can play an important role in algae-pollutant interactions (Kruk et al. 2010; Pomati and Nizzetto 2013). In the case of copper, the expectations of

morphological traits as predictors of any type of stress response were met as biovolume, surface-to-volume ratio and cell length predicted the response of diatom density, photosynthetic efficiency, fatty acid and EPS production to copper. The case of atrazine however demonstrated that relationships between morphological traits and numerical and functional response are stressor-specific. As common chemical stressors in coastal and estuarine environments, the effects of atrazine and other photoinhibiting herbicides on primary producers in these ecosystems have been extensively analysed (DeLorenzo et al. 1999, 2001; Zhang et al. 2008), but mainly in terms of their effects on the density and not on the functions sustained by the species concerned, and in relation to taxonomy rather than species traits. Mixotrophy is common in benthic diatoms (Tuchman et al. 2006), and the few approaches which related the effects of photoinhibiting herbicides to mixotrophy suggest the numerical response of diatoms to these stressors can be modulated by their trophic mode (Debenest et al. 2009; Larras et al. 2014). Here, mixotrophy predicted the numerical response of diatoms, but not the changes in any of their three effect traits under atrazine. Estimating the effects of photoinhibiting stress on ecosystem functions sustained by primary producers is therefore less straightforward than under metal stress, since the numerical and functional response to photoinhibiting stress are not driven by the same traits and mixotrophy cannot be quantified as easily as morphological traits. Nevertheless, the capacity for mixotrophic growth can be obtained from the literature for many taxa (Hellebust and Lewin 1977; Tuchman *et al.* 2006). Our study indeed corroborates literature reports for *Cylindrotheca closterium* (Vanelslander et al. 2009), which was also the most mixotrophic species in our experiments.

Relating traits to stress response should be done by linking traits to groups of stressors with similar modes of action, to generalize the trait-sensitivity relation for distinct types of stressors (Baird and Van den Brink 2007). Atrazine blocks the electron transport chain of Photosystem II (PSII), resulting in the excitation energy generated in PSII being lost as heat or fluorescent light instead of being used for CO_2 assimilation in the Calvin cycle (Dorigo and Leboulanger 2001). In this respect, atrazine is representative for more than half of the currently available herbicides, whose mode of action consists of inhibiting the electron flow in PSII (Fedtke 1982; Stenersen 2004). Mixotrophy as a response trait predicted diatom densities, but not functioning under atrazine exposure, and those species which could best maintain their cell densities and photosynthetic efficiency under atrazine were most sensitive in their EPS and fatty acid production. These contrasting effects of atrazine on density and functioning imply that at herbicide stress levels where species densities are not yet impacted, sediment stabilization and energy flux to higher trophic levels would already be affected, with concomitant knock-on effects on other sediment-associated organisms and the food web in intertidal sediments (Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000; Mason et al. 2003).

For metal pollution, the functional consequences seem at a first sight smaller: the numerical and functional response to copper were positively correlated, meaning that species that were little affected in their density also performed best at maintaining their contribution to ecosystem functioning. Copper, like other metals, does not only inhibit algal photosynthesis but also leads to the formation of reactive oxygen species, which can damage cell membranes, plastids and other intracellular structures (Rijstenbil et al. 1994; Cid et al. 1995; Miao et al. 2005; Knauert and Knauer 2008). Metal tolerance has been related to cell size, with smaller species having a larger surface-to-volume ratio and thus being more prone to be adversely affected by metal pollution (Miao et al. 2005; Quigg et al. 2006). Since the same traits that predicted the numerical and functional response to copper in this study also explain tolerance to pollution by other metals (Miao et al. 2005; Lahive et al. 2011), large, metal-resistant species could potentially sustain ecosystem functioning under metal pollution. However, large cell size is correlated with other traits that can reduce fitness, such as lower nutrient acquisition, slower growth and longer generation times (Litchman and Klausmeier 2008; Litchman *et al.* 2015). If simultaneously with metal pollution diatoms are subjected to other types of stress that favour traits such as small cell size or short growth cycles, the competitive advantage and the capacity of large-bodied species to sustain ecosystem functioning will be limited. Using the response-effect-trait framework to estimate the effects of environmental stress on ecosystem functioning will depend on whether stressors target a specific metabolic function (e.g. herbicides), with stress tolerance linked to traits that do not affect general fitness (e.g. mixotrophy). When stressors impact on a broad array of metabolic functions (e.g. metals), and stressor effects depend on traits linked to fitness (e.g. morphological traits), the predictive power of the response-effect-trait framework in a natural environment will be limited.

The above conclusions should be interpreted within the context of our study's experimental design. By using xenic diatom cultures, we did not control the growth of bacteria in our experimental microcosms. Bacteria can degrade organic substances such as the glucose used in our mixotrophy experiments (Grossart and Ploug 2001; Bruckner et al. 2008), which can stimulate diatom growth by providing CO_2, organic and inorganic nutrients (Bruckner et al. 2008; Fouilland 2012; Novoveská et al. 2016). We however tested mixotrophy by comparing diatom growth in treatments containing respectively atrazine and glucose as well as atrazine only. Photosynthesis was thus blocked in both types of treatments and any nutrients or CO_2 released by bacteria should have had little effect on diatom growth.

We analysed photosynthetic efficiency as well as EPS and fatty acid production as effect traits with regard to their importance for ecosystem functioning (primary production, sediment stabilization, energy transfer to higher trophic levels). However, maintaining a high

photosynthetic efficiency will also favour tolerance to most types of growth-limiting stress (Dummermuth et al. 2003; Skene 2004; Wu et al. 2007). EPS production can serve as a protective mechanism against various forms of abiotic stress (Liu and Buskey 2000; Staats et al. 2000a; Levy et al. 2007), which can however not be maintained when photosynthesis is inhibited (Wang et al. 1997; Staats et al. 2000b; Mason et al. 2003). Oxidative stress resulting from metal exposure results in the damage of fatty acid rich structures such as cell membranes due to lipid peroxidation (Li et al. 2006), and a high fatty acid production could help diatoms to better tolerate the damage to cellular structures caused by heavy metal stress. The same traits may drive both the contribution to functioning and the ability to persist under stress, and overlaps between response and effect traits should be considered when predicting ecosystem functioning under environmental change (Díaz et al. 2013; Karp et al. 2013).

Lastly, the intraspecific variability of traits should be considered when predicting stress response (Albert et al. 2010; De Laender et al. 2014). Size variation is inherent to the diatom life cycle, which consists of a prolonged vegetative stage lasting months to years, where diatom cells multiply mitotically and gradually reduce in size, followed by a short sexual stage resulting in enlarged cells which then restart a new round of vegetative multiplication (Lewis 1984; Chepurnov *et al.* 2008). We observed little changes in morphological traits under stress exposure, but our experiments lasted only a limited number of vegetative growth cycles and hence negligible size reduction, implying that potential changes in diatom morphology might be driven by evolutionary processes rather than physiological acclimation. Diatoms can shift their trophic mode with almost immediate effect (Lewin and Hellebust 1975), whereas significant changes in morphology occur over longer time spans which makes trait variability more likely in long-term experiments and in scenarios of constant stress exposure.

In conclusion, this study has identified morphology and mixotrophy as key response traits driving algal stress response, adressing current gaps in the knowledge on traits which still limit the application of trait-based frameworks (Lavorel et al. 2013), and contributing to the understanding of environmental change-driven shifts in the densities of marine primary producers (Moran et al. 2010; Litchman et al. 2015). More importantly, it has been shown that chemical pollution causes not only a numerical, but also a functional response by directly altering the effect traits of microalgae. Compared to changes in species densities and concomitantly in community structure, such direct stressor impacts on organisms' contribution to functioning have received little attention to date (Relyea and Hoverman 2006; Rohr et al. 2006; De Laender et al. 2016). In the context of multiple human impacts (Lotze et al. 2006; Crain et al. 2008), the different relations of numerical and functional responses under pesticide and metal stress highlight the wide range of possible outcomes of response-effect trait frameworks. Accounting for both the numerical and

functional response of stressed organisms could extend the predictive capacity of trait-based frameworks under environmental change.

Chapter 4: Facilitation and tolerance explain the diversity effect on functioning in stressed diatom communities

Abstract

The relation between biodiversity and ecosystem functioning depends on complementarity and dominance effects, but the ecological processes driving complementarity and dominance under anthropogenic stress remain largely unknown.

We exposed two benthic diatom communities to metal and pesticide stress (copper and atrazine), and measured complementarity and dominance effects on biomass yield under stressed and unstressed conditions. Next, we analysed if complementarity under stress was driven by trait-independent complementarity (affecting species independent of their biomass yield) or trait-dependent complementarity (differentially affecting high- and low-yield species). Last, we tested if the production of extracellular polymers as a facilitative mechanism as well as the species' stress tolerance and capacity for mixotrophic growth were ecological processes that explained complementarity and dominance under stress.

The net biodiversity effect on diatom biomass yield was consistently positive, and was mainly caused by trait-independent complementarity, which was higher in diatom communities stressed by copper (both communities) and atrazine (one community) than in unstressed communities. Stimulation by stress of the diatom's extracellular polymer production predicted, in part, these increases of complementarity under stress.

Complementarity in communities stressed by the atrazine, a photosynthesis inhibitor, was driven by the presence of mixotrophic species, which can grow on organic substances. Under copper stress, complementarity in one community was driven by the species which were least tolerant to the metal in monoculture. These species were characterised by low biomass yields, resulting in a negative trait-dependent complementarity, reducing the net biodiversity effect under metal stress.

Biodiversity effects on diatom biomass were caused by trait-independent complementarity effects, which increased under stress and were driven by extracellular polymer production, a 'sleeping' facilitative mechanism stimulated by stress. The degree to which diatom species benefited from complementarity depended on their trophic mode and stress tolerance. When complementarity favoured the growth of sensitive but low-yield species, the net biodiversity effect on diatom biomass yield decreased under stress.

4.1. Introduction

Understanding the influence of biodiversity on ecosystem functioning is a key challenge, as global change is causing unprecedented rates of biodiversity change (Pereira et al. 2010, Dornelas et al. 2014, Gonzalez et al. 2016). A large amount of empirical research has documented in most cases positive effects of biodiversity on ecosystem processes (most commonly biomass yield, Balvanera et al. 2006, Cardinale et al. 2011, Naeem et al. 2012), with results being largely consistent across trophic levels and ecosystem types (Cardinale et al. 2006, Griffin et al. 2013).

Anthropogenic stressors are a main driver of biodiversity change (Wilcove and Master 2005, Malaj et al. 2014), and can substantially affect biodiversity-ecosystem functioning relationships (Steudel et al. 2012, Baert et al. 2016). An increasing number of studies have tested the biodiversity effect on ecosystem functioning under stressful conditions, but these have yielded contrasting results: clearly positive effects in Mulder et al. (2001), Ives and Cardinale (2004), Solan et al. (2004), Zavaleta and Hulvey (2004), Larsen et al. (2005), McMahon et al. (2012), Steudel et al. (2012), Mensens et al. (2015), Baert et al. (2016) and neutral or even negative diversity effects in Jonsson et al. (2002), Petchey et al. (2002c), Smith and Knapp (2003), Jiang (2007), Jiang et al. (2008), Roger et al. (2012). This variability of results calls for more mechanistic studies on how stress affects diversity effects on functioning, relating ecological processes to stress-induced alterations of biodiversity effects on function (Naeem 2008, Solan et al. 2009, O'Connor et al. 2015, Baert et al. 2016).

Dominance (dominance of communities by high- or low-yield species) and complementarity (better performances of species in community due to mechanisms such as resource partitioning or facilitation) are the main diversity effects which cause communities to perform differently than expected from monocultures of the constituent species (Loreau and Hector 2001, Fox 2005). Dominance and complementarity mostly operate simultaneously (Loreau et al. 2001). Consequently, which effect drives the diversity-functioning relation and how both effects are affected by anthropogenic stress is actively debated (Cardinale et al. 2007, Fargione et al. 2007, Jiang et al. 2008, Loreau 2010, Wang et al. 2013, Baert et al. 2016, Hodapp et al. 2016). Complementarity and dominance effects can drive ecosystem functioning under stressful conditions through stress-induced increases of facilitation (Wang et al. 2013) and when communities are dominated by stress-tolerant species with above-average contribution to functioning (Baert et al. 2016), respectively. Even though still scarce at the moment, stressor-induced changes in dominance and complementarity are increasingly tested for (Wang et al. 2013,

2138 Baert et al. 2016), using analytical methods based on the covariance between species performance
2139 in a community and in monoculture (Loreau and Hector 2001, Fox 2005).

2140 On itself, quantifying complementarity and dominance does not offer direct mechanistic insight
2141 into the ecological processes driving stress effects on biodiversity-ecosystem functioning
2142 relationships. That is because complementarity and dominance *sensu* Fox (2005) or Loreau and
2143 Hector (2001), who refer to complementarity and selection), are statistical effects that can result
2144 from multiple ecological processes (Cardinale et al. 2011, Tilman et al. 2014). One such process
2145 is facilitation, which can drive complementarity in legumes, insects and microalgae (Cardinale et
2146 al. 2002, Temperton et al. 2007, Vanelslander et al. 2009). Further tests of facilitative mechanisms
2147 as drivers of complementarity are however lacking and there is no direct evidence attributing
2148 changes of complementarity effects under stress to changing facilitative mechanisms.

2149 Here, we quantify complementarity and dominance effects on the biomass yield of two benthic
2150 diatom communities under chemical stress, and compare these with effects determined in
2151 unstressed controls. We use benthic diatom communities as an experimental model because they
2152 are the main primary producers in many intertidal, soft-sediment habitats, and diatom biomass
2153 production is crucial as it represents the basis of the food web in these systems (Montagna et al.
2154 1995, Underwood and Kromkamp 1999, Tang and Kristensen 2007). We use copper and the
2155 herbicide atrazine as model stressors, the former being a common pollutant in coastal ecosystems
2156 due to urban and industrial runoff (Kennish 1996, Murray-Gulde et al. 2002), and the latter
2157 representing one of the most-used agricultural herbicides worldwide (Graymore et al. 2001,
2158 Chirnside et al. 2007).

2159 We then test if complementarity under stress was driven by trait-independent complementarity
2160 (affecting diatom species comparably) or trait-dependent complementarity (differentially
2161 affecting species with high or low biomass yields). Benthic diatoms produce large amounts of
2162 extracellular polymeric substances (EPS, Decho 1990, Underwood and Paterson 2003). These
2163 organic exudates have been linked to metal tolerance in diatom monocultures (Pistocchi et al.
2164 1997, Levy et al. 2007) and can cause facilitation in diatom communities containing mixotrophic
2165 species (Vanelslander et al. 2009). We therefore analyse if the release of EPS could predict
2166 complementarity in stressed diatom communities, and test if stressor-induced changes in
2167 complementarity were related to the species' stress tolerance in monoculture and the presence of
2168 mixotrophic species.

2169

4.2. Methods

Experimental organisms and culture conditions. The two diatom communities (hereafter community A and B) were each composed of six benthic diatom species (Addendum III Table S1) representing the most abundant genera observed at two muddy intertidal sites at the Paulina intertidal flat (SW Netherlands, 51° 21'N, 3°43'E). For this experiment, all species were obtained from the culture collection of the Protistology and Aquatic Ecology Research Group (UGent) (http://bccm.belspo.be). Diatoms were maintained in a climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons $m^{-2}s^{-1}$, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients (Guillard 1975).

Atrazine and Copper experiments. Diatoms were exposed to 0 (control), 200 and 500 µg/l (hereafter 'low' and 'high') atrazine and copper respectively. In a previous experiment, these concentrations resulted on average in a 50% and 90% reduction of diatom monoculture yield (Chapter 3). Atrazine treatments were prepared from a stock solution obtained by dissolving 50 mg technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure, Sigma-Aldrich Chemie Gmbh, Munich, Germany) in 10 ml acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.01% acetone in the treatments. An acetone control treatment of 0.01% acetone was included and compared to an acetone-free control to test for carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a $Cu[II]Cl_2$ solution, analytical grade; VWR International) was spiked directly into the culture medium before exposure of the diatoms. F/2 culture medium for the copper experiments was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The resulting atrazine and copper concentrations were determined by respectively GC-MS and graphite furnace AAS analyses (Addendum III Table S2). All experiments were run in tissue culture flasks (Greiner BioOne, CELLSTAR® TC, 175 cm^2 growth surface), with nine replicates per treatment (three replicates in the monoculture treatments). Each microcosm was inoculated with a total cell density of 5000 diatom cells/ml (5000 cells/ml for each species in monoculture, between 800-850 cells/ml per species in communities). Microcosms were inoculated with cells from exponentially growing cultures and were incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons $m^{-2}s^{-1}$. Culture medium of all treatments was refreshed after eight and 15 days, and experiments were terminated after 25 days.

Diatom biomass. Diatom biomass was quantified as biovolume, and was calculated from cell densities and mean cell biovolume per species, using measured linear dimensions and formulas representing the closest approximation of geometric shape for each genus Hillebrand *et al.* (1999). Cell densities were obtained at day 0, 2, 5, 10, 15, 20 and 25 of the experiment by magnifying and

photographing (x100) an area of 0.66 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11 digital camera). Cell numbers in the photographs were subsequently determined by using the cell counting plugin within the ImageJ software (Schneider et al. 2012), where cells are counted digitally by placing marks of different colours on cells of the respective species, with ImageJ automatically generating a list of counted cells per species. Cell counts were converted into cell densities in cells/ml. All further analyses use the biomass on day 15 (late exponential growth phase).

EPS production. Diatom biofilms are characterized by a high production of EPS (Underwood and Paterson 2003), which represent a mix of organic compounds consisting mostly of polysaccharides, but also proteins and nucleic acids (Baines and Pace 1991). These organic compounds play an important role for sediment stabilization in muddy intertidal areas (Decho 2000, Underwood and Paterson 2003). The release of organic compounds into the medium has also been identified as driver of complementarity effects in benthic diatoms (Vanelslander et al. 2009). Furthermore, benthic diatoms can modulate their EPS production in response to abiotic stress, notably copper (Pistocchi et al. 1997, Levy et al. 2007). We therefore tested whether the EPS released by diatoms into the medium could predict potential changes of complementarity under chemical stress. EPS were measured by spectrophotometry after 15 days, in three replicates of 10 ml suspended diatom culture per treatment. The suspended diatom cultures were centrifuged for 15 min at 15 °C and 3500xg. The supernatant yielded the soluble EPS fraction, which was left to precipitate overnight in 30 ml cold ethanol (98%), and subsequently centrifuged for 15 min at 15 °C and 3500xg. The pellet was dried under a flow of nitrogen and resuspended in 1 ml of 1.5% NaCl. Samples of 200 µl of this suspension per replicate were used for the EPS analysis. EPS were measured according to a modified version of the phenol/H_2SO_4 assay by Dubois et al. (1956). In 24-well-plates, 200 µl phenol (5%, w / v in distilled water) and 1 ml concentrated H_2SO_4 were added to 200 µl sample. The mixture was shaken, incubated for 20 min and the absorbance measured at 488 nm using a Victor^3 multilabel reader (Perkin-Elmer). EPS concentrations were quantified with a function obtained from a linear regression of known concentrations of glucose standards (ranging from 5 to 400 µg/ml).

Data analysis. For every treatment, we partitioned the net biodiversity effect on diatom biomass into dominance, trait-independent complementarity and trait-dependent complementarity, using the method developed by Loreau and Hector (2001) and later extended by Fox (2005, Equation 1).

$$\begin{aligned}\Delta\mathrm{Y} = \quad & N \times \mathrm{cov}\left(\mathrm{M}_i, \frac{\mathrm{RY_i}}{\mathrm{RYT}} - \mathrm{RY_{E}}i\right) \\ & + \mathrm{N} \times (\overline{\Delta\mathrm{RY}}) \times (\bar{M}) \\ & + N \times \mathrm{cov}\left(\mathrm{M}_i, \mathrm{RY}_i - \frac{\mathrm{RY_i}}{\mathrm{RYT}}\right)\end{aligned} \qquad [1]$$

ΔY is the net biodiversity effect on diatom biomass yield, i.e. the difference between the observed community yield and the community yield that is expected from the monoculture yields of the component species. M_i is the mean monoculture yield of species *i.* $\mathrm{RY_i}$ is the observed relative yield of species *i with* $\mathrm{RY}_i = \frac{O_i}{M_i}$, where O_i is the observed biomass of species *i* in the community. RYT is the relative yield total with $\mathrm{RYT} = \sum_{\mathrm{i}=1}^{\mathrm{N}} \mathrm{RY_i}$ where N is the total number of species in the community (six). $\frac{\mathrm{RY}_i}{\mathrm{RYT}}$ is the observed frequency of species *i* in the community, i.e. its share of the relative yield total. $\bar{M}$ is the average monoculture yield of all species. $\mathrm{RY_{E}}i = \frac{O_{i,t=0}}{O_{t=0}}$ is the expected relative yield of species *i* in the community, where $O_{i,t=0}$ and $O_{t=0}$ are the observed biomass of species *i* and the observed community biomass at the start of the experiment, respectively, with $\sum_{i=1}^{N} \mathrm{RY_{E}}i = 1$. The mean deviation in relative yield $\overline{\Delta\mathrm{RY}}$ was calculated as the mean difference in observed relative yield and expected relative yield $\overline{\Delta\mathrm{RY}} = \frac{1}{N} \times \sum_{i=1}^{N}(\mathrm{RY}_i - \mathrm{RY_{E}}i)$.

The first term on the right-hand side of equation 1 is the 'dominance effect' (Fox 2005), which is the covariance between the species' monoculture yields and the difference between their observed frequencies in community and expected relative yields. The dominance effect is positive when species with high monoculture yields also reach high yields in community at the expense of low-yield species. It is negative if communities are dominated by low-yield species at the expense of high-yield species. Mechanisms for this effect include interspecific competition for shared resources.

The second term is the trait-independent complementarity (TIC) effect (Loreau and Hector 2001, Fox 2005), which quantifies the extent to which the species' observed yields in community deviate from the expected yield, but in a way that is independent of the individual species' monoculture yields. Trait-independent complementarity is positive when growth in community rather than monoculture increases the yield of species, independent of their monoculture yield and not at the expense of other species. Mechanisms underlying this effect can be niche partitioning or facilitation.

The third term is the trait-dependent complementarity (TDC) effect (Fox 2005), which is the covariance between the species' monoculture yield and the deviation of their observed frequency

in community from their observed relative yield. Trait-dependent complementarity effects are observed when growth in community rather than monoculture increases the functioning of species with high or low monoculture yields, but not at the expense of other species. Trait-dependent complementarity can be positive if, for example, species with high monoculture yield are facilitated by species with low monoculture yield, but not vice-versa. Trait-dependent complementarity effects can become negative if low-yield species but not high-yield species benefit from facilitation when being grown in community.

To analyse if the net biodiversity effect, DOM, TIC and TDC changed under atrazine and copper stress, we used generalised least squares models with the biodiversity effects as response variables and the treatment type as categorical predictor (Equations 2-5).

$$\Delta Y = a + b \times T \quad [2]$$

$$DOM = a + b \times T \quad [3]$$

$$TIC = a + b \times T \quad [4]$$

$$TDC = a + b \times T \quad [5]$$

ΔY is the net biodiversity effect on diatom biomass yield, DOM is the dominance effect, TIC and TDC are trait-independent and trait-dependent complementarity, a is the intercept, T is the treatment type (control, low and high atrazine and copper) with the control set as reference level, b is the slope, i.e. the effect of the treatment type on each biodiversity effect.

Next, we tested the role of EPS as a potential driver of complementarity in diatom communities by fitting generalized least squares models to trait-independent complementarity as response variable and diatom EPS production as predictor (Equation 6).

$$TIC = a + b \times EPS \quad [6]$$

TIC is trait-independent complementarity, a is the intercept, EPS the amount of EPS produced per unit diatom biomass and b is the slope, i.e. the effect of EPS production on TIC. Models were fitted separately for each diatom community and stressor.

In a last step, we tested if changes in trait-dependent complementarity were driven by species with a low or high monoculture yield and/or stress tolerance benefiting most from being grown in community. We fitted generalized least squares models to the monoculture yield and stress tolerance of the diatom species as predictors, and the deviation of their observed frequency in community from their observed relative yield as response variable (Equation 7)

$$\mathrm{RY}_{i,j} - \frac{\mathrm{RY}_{\mathrm{i,j}}}{\mathrm{RYT}_j} = a + b \times \mathrm{M}_{i,j} + c \times \frac{\mathrm{M}_{i,j}}{\mathrm{M}_{i,j=0}} \qquad [7]$$

The fraction on the left-hand side of Equation 7 is the deviation of the observed frequency in community from the observed relative yield of species *i* at stress level *j*. This term (hereafter relative performance) quantifies the extent to which each species was effected by trait-dependent complementarity (see Equation 1). $\mathrm{M}_{\mathrm{i,j}}$ is the monoculture yield of species *i* at the stress level *j*, $\frac{\mathrm{M}_{i,j}}{\mathrm{M}_{i,j=0}}$ is the tolerance of species *i*, quantified is the ratio of the monoculture yield at stress level *j* and the monoculture yield in the control (*j*=0), a is the intercept, b and c are the slopes, i.e. the relation between the species' yield and tolerance in monoculture and the deviation of their relative performance in community. If the monoculture yield and tolerance were correlated (correlation factor > 0.5), models were fitted separately for both predictors. Models were fitted separately for each diatom community and stressor.

For all least squares model fits, normality and homogeneity of model residuals were inspected by evaluation of quantile-quantile plots and Shapiro-Wilk's test, and by Levene's test and plotting residuals versus explanatory variables respectively. If normality was violated (for model fits in equation 7 only), data were log-transformed. If homogeneity was violated, the model was refitted using a variance structure allowing different variances per treatment T ('weights = varIdent(form ~ 1 | T' in the R package nlme (Pinheiro et al. 2016) and homogeneity was evaluated again. By means of likelihood ratio testing, the significance of these structures was tested (alpha = 0.05). All calculations were performed in R 3.0.1. using RStudio (R Development Core Team 2016).

4.3. Results

Effects of stress on dominance and complementarity. The net biodiversity effect was negative in community A under control conditions, but became positive at high copper and both atrazine levels. In community B, the net biodiversity effect was positive in all treatments, i.e. the biomass yield of diatoms in community was higher than that expected from the component species' monocultures (Addendum III Fig. S1, Table S3). Across all treatments of community A, the net biodiversity effect was driven by positive complementarity effects and negative dominance effects, , whilst in community B complementarity was the main effect causing the yield of diatom communities to deviate from that expected from their monocultures (Fig. 4.1).

In community A, all tested levels of atrazine and copper altered the two biodiversity effects (Fig. 4.1A), driving the negative dominance effect closer to zero, except for low copper levels which reduced the dominance effect, and further increasing the positive complementarity effect (Fig. 4.1A, Addendum III Tables S4 and S5).

In community B, both stressors also changed complementarity (Fig. 4.1B). However, in contrast to community A, these changes were negative at high stress levels: the large and positive complementarity effect was reduced by high levels of both stressors, but was still well above zero (Fig. 4.1B, Addendum III Table S5). The small and negative dominance effect was not affected by stress except for the low atrazine level, which drove the dominance effect closer to zero (Fig. 4.1B, Addendum III Table S4).

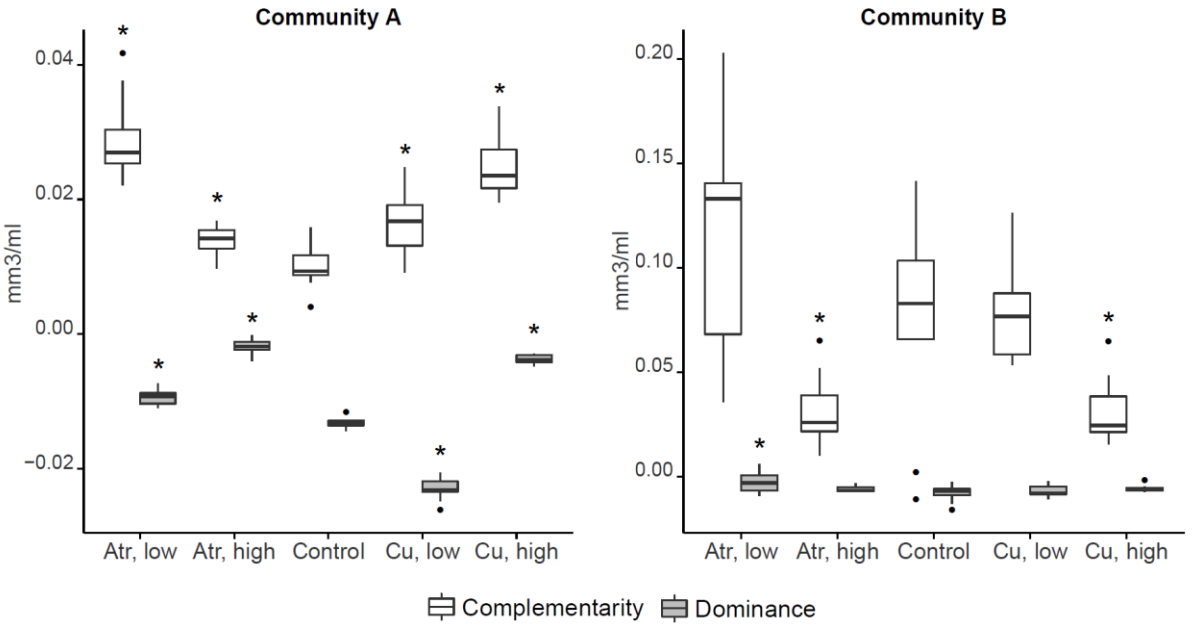


Fig. 4.1: Deviation in the observed from the expected yield (in mm3/ml) of diatom communities that could be attributed to either dominance and complementarity effects. Diatom communities were exposed to low and high (200 and 500 µg/L) concentrations of copper (Cu) and atrazine (Atr). Asterisks (*) indicate significant differences in the dominance and complementarity effect from the respective control. Complementarity is indicated as the sum of trait-independent and trait-dependent complementarity effects.

Complementarity effects in community A were essentially composed of trait-independent complementarity (TIC, Fig. 4.2A), which was consequently always positive and positively affected by all stress levels (Fig. 4.2A, Addendum III Table S5). The contribution of trait-independent and trait-dependent complementarity (TDC) were more comparable in community B than in community A. Complementarity effects in community B were mostly composed of TIC (control and atrazine treatments), and by both TDC and TIC (copper treatments, Fig. 4.2B). Interestingly, low stress had no (atrazine) or negative (copper) effects on TDC and positive (copper) or no (atrazine) effects on TIC, while high levels of both stressors had negative effects on TDC (Fig. 4.2B,

Addendum III Table S5). Thus, negative effects on TDC were partly offset by positive effects on TIC when stress was low, but not when stress was high.

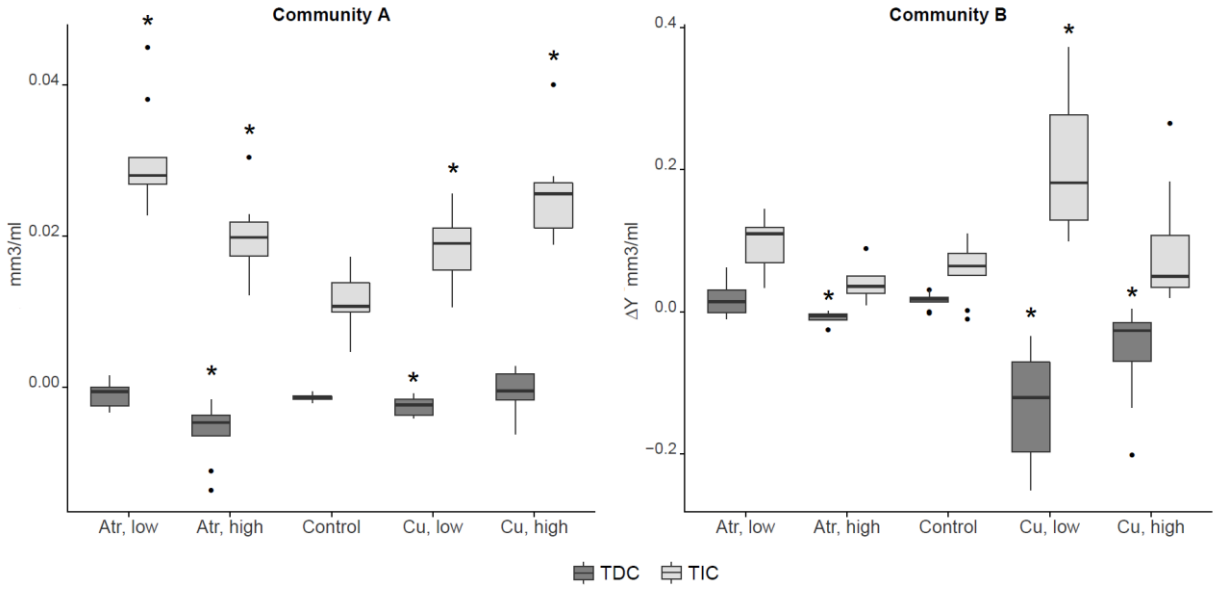


Fig. 4.2: Deviation in the observed from the expected yield (in mm3/ml) of diatom communities that could be attributed to either trait-independent complementarity effects (TIC) and trait-dependent complementarity effects (TDC). Diatom communities were exposed to low and high (200 and 500 µg/L) concentrations of copper (Cu) and atrazine (Atr). Asterisks (*) indicate significant differences in TIC and TDC from the respective control..

Linking stress effects on trait-independent complementarity to EPS production. The release of EPS predicted the increase of TIC in community A under atrazine and in community B under copper (Fig. 4.3). In community A, diatoms increased their EPS production at low atrazine and copper stress. EPS secretion and TIC were positively related when diatoms were exposed to atrazine (Fig. 4.3A, Addendum III Table S6), but not to copper. In community B, diatoms increased their EPS production at low copper, but not atrazine stress. EPS and TIC were not related under atrazine stress, but the amount of EPS produced by the diatoms positively affected TIC in communities exposed to copper (Fig. 4.3B, Addendum III Table S6).

Linking stress effects on trait-dependent complementarity to stress tolerance and monoculture yield. In community A, the relative performance of diatoms (the deviation of the observed frequency in community from the observed relative yield, the main component of TDC) was not related to stress tolerance or monoculture yield (Fig. 4.4 A1-A2, Addendum III Table S7). Diatoms in this community differed in both their stress tolerance and monoculture yield (see x-axes in Fig. 4.4 A1-A2), but growth in community did not benefit species with either above- or below-average stress tolerance or monoculture yields.

In community B, atrazine tolerance and monoculture yield also did not predict the relative performance of diatoms in communities exposed to atrazine (Fig. 4.4 B1-B2, Addendum III Table S7). Under copper stress, the relative performance of diatoms was negatively related to both their copper tolerance and monoculture yield (Fig. 4.4 B1-B2, Addendum III Table S7). These negative correlations were caused by *Amphora lineolata*, *Gyrosigma* sp. 1 and *Navicula digitoradiata*, which were the least copper-tolerant and lowest-yield-species within community B (Fig. 4.4 B1-B2). Despite their low monoculture yields and stress tolerance, *N. digitoradiata, Gyrosigma* sp. 1, and *A. lineolata* however showed the highest relative performance when grown in community (Fig. 4.4 B1-B2). In community B trait-dependent complementarity under copper stress thus benefited the species which were most sensitive to the metal, but also had the lowest biomass yields, which led to a decline of total complementarity and ultimately caused a decrease of the net biodiversity effect on diatom biomass under copper stress.

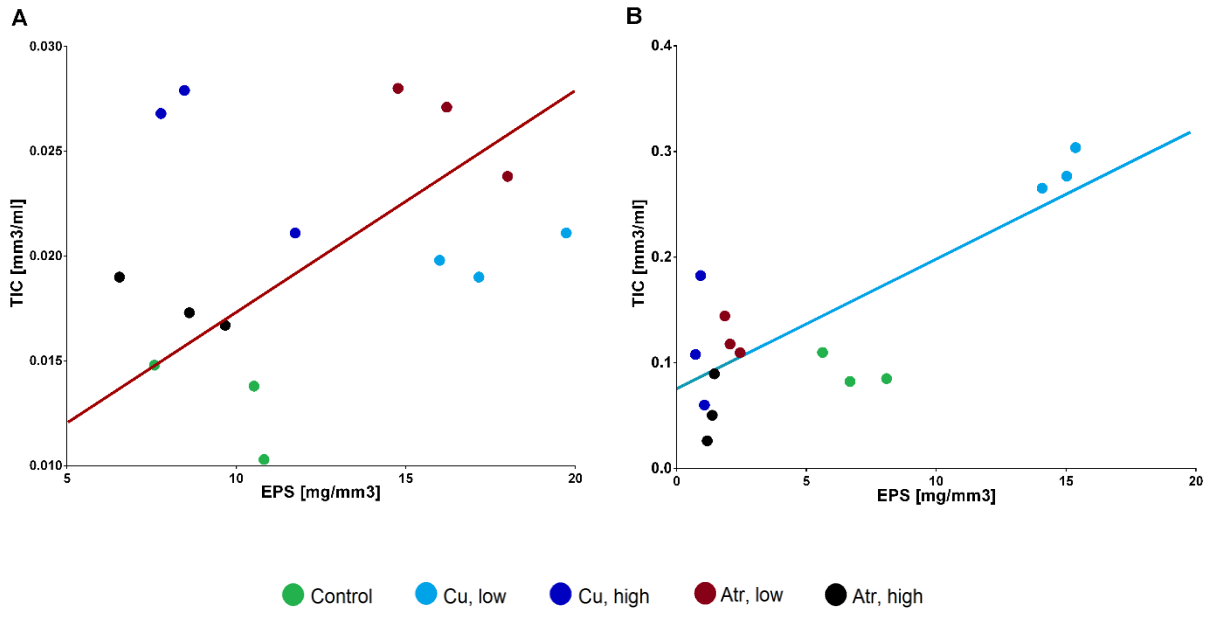


Fig. 4.3: Trait-independent complementarity (TIC) as a function of the release of extracellular polymeric substances (EPS) by two benthic diatom communities (panels A and B) exposed to low and high (200 and 500 µg/L) concentrations of copper (Cu) and atrazine (Atr). Regression lines show significant relations between EPS and TIC in diatom communities when exposed to copper (blue line) and atrazine (red line). Regressions were fitted over three treatments (control, 200 and 500 µg/L) for each stressor.

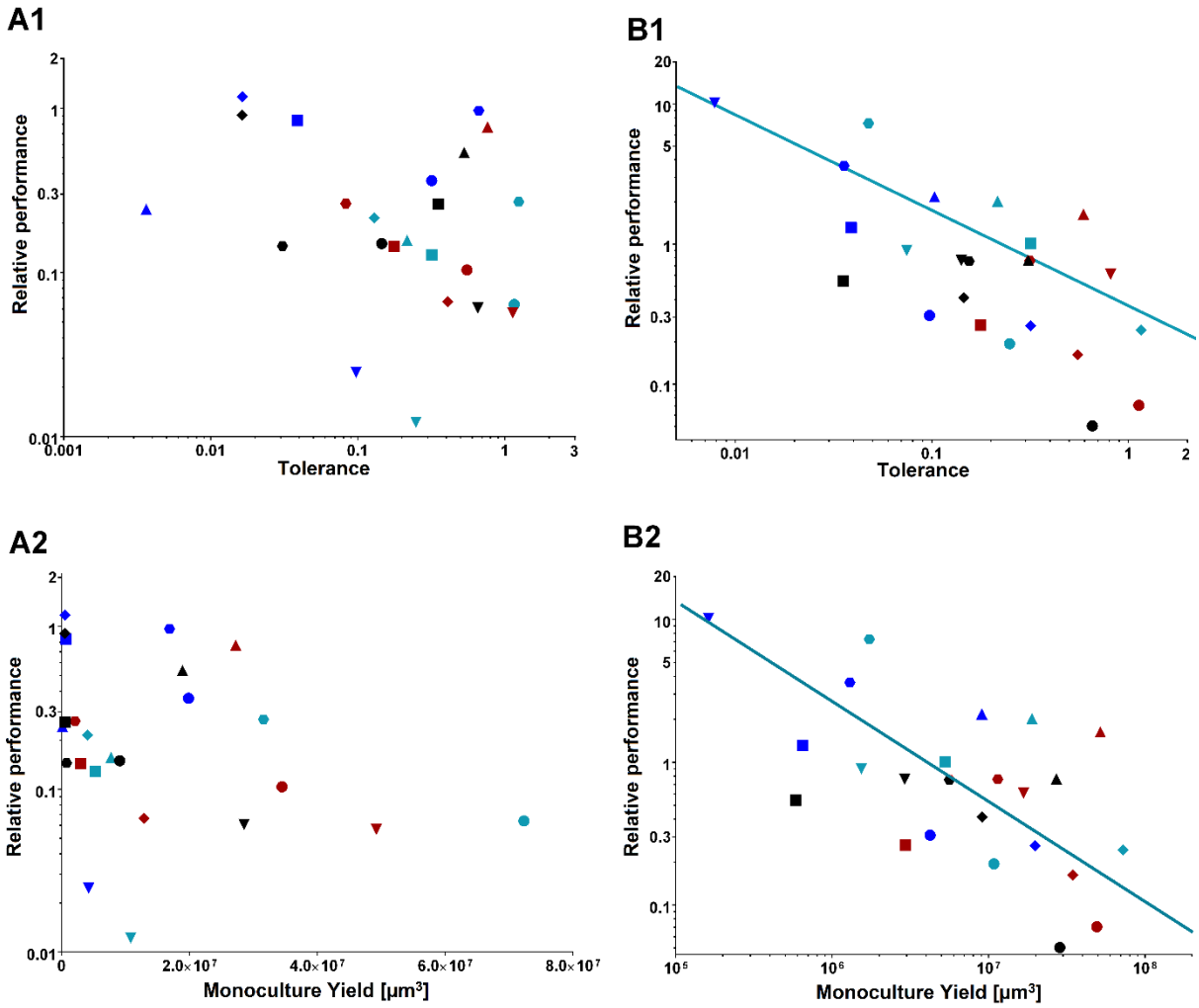


Fig. 4.4: Relative performance of diatom species in community as a function of their stress tolerance and biomass yield in monoculture. The panels A1 and B1 show the relative performance of diatom species (calculated as the deviation of the observed frequency (RY/RYT) from the observed relative yield (RY)) as a function of stress tolerance (calculated as the ratio of the monoculture yield at each stress level and in the control) in community A (panel A1) and B (panel B1). Panels A2 and B2 show the relative performance as a function of monoculture yield for diatoms in community A (panel A2) and B (panel B2). Stress levels are low and high (200 and 500 µg/L) atrazine (Atr) and copper (Cu). Regression lines show significant relations between the deviation of the species' relative performance in community and their stress tolerance and biomass yield in monoculture. Data are shown on a log scale, as models were fitted to log-transformed data.

4.4. Discussion

The outcome of our experiment shows complementarity rather than dominance effects as the main driver of stress-induced increases in the biodiversity effect on diatom biomass production. The diatoms' production of extracellular polymers predicted, in part, increases in trait-

independent complementarity under stress. These increases could however be offset by a negative trait-dependent complementarity: when low-yield species were least stress-tolerant in monoculture but benefited most from complementarity, the diversity effect on the biomass yield of stressed diatom communities was reduced.

Diatom biomass production is crucial for the functioning of muddy intertidal systems, since benthic diatoms represent the main primary producers and thus the basis of the food web in these areas (Underwood and Kromkamp 1999, Tang and Kristensen 2007). Here, the biodiversity effect on diatom biomass production was consistently positive in community B, and turned from negative to positive when community A was exposed to atrazine and high copper stress (Appendix III Fig. S1). In both communities, complementarity was the main driver of the positive biodiversity effect under stress (Fig. 4.1), highlighting the value of producer diversity for maintaining biomass production at the basis of intertidal food webs under stressful conditions. Our results corroborate previous studies which found complementarity to drive positive diversity effects on microalgal biomass (Vanelslander et al. 2009, Cardinale 2011, Stockenreiter et al. 2012, Schabhüttl et al. 2013), as well as experiments which found positive complementarity effects to offset negative dominance effects under stress (Reusch et al. 2005, Parker et al. 2010, Hughes et al. 2010, Wang et al. 2013).

Complementarity effects increase under stress if facilitation, one of the mechanisms potentially leading to complementarity, becomes more important under harsh environmental conditions (Mulder et al. 2001, Wang et al. 2013, Hodapp et al. 2016). To date, ecophysiological evidence for potential facilitative mechanisms under stress is however scarce. The release of organic exudates has been identified as a facilitative mechanism driving complementarity effects in diatom biofilms under unstressed conditions (Vanelslander et al. 2009). The release of extracellular polymers also represents a common stress response in diatom biofilms: diatoms can increase their EPS production in response to nitrogen depletion, low temperatures or other unfavourable abiotic conditions (Staats et al. 2000a, Wolfstein and Stal 2002). EPS production has furthermore been linked to protection from desiccation, physical disturbance and resistance to toxic chemicals (Decho 1990, Levy et al. 2007, Gerbersdorf et al. 2009b) as long as stress levels do not inhibit photosynthesis and thus carbon supply for EPS biosynthesis (Wang et al. 1997, Staats et al. 2000b).

In the presence of atrazine, diatoms in community B did not increase their EPS production and there was no relation between EPS production and complementarity (Fig. 4.3 B). In community A however, a stimulated EPS production at low atrazine levels predicted the increase of complementarity in diatom communities exposed to the herbicide (Fig. 4.3 A). Atrazine blocks the

electron transport chain of Photosystem II (PSII, Dorigo and Leboulanger 2001). Some diatom species can however reduce their dependency on photosynthesis and thus their sensitivity to PSII inhibitors by mixotrophic growth, i.e. the uptake of organic substances, if such substances are present in sufficient amounts in the medium (Debenest et al. 2009, Larras et al. 2014). Amongst the diatom species in community A, *Cylindrotheca closterium* has been shown to increase its growth in response to organic exudates by other diatom species (Vanelslander et al. 2009), and *C. closterium*, *Nitzschia* sp. 1 and *Nitzschia acicularis* can grow in the presence of atrazine when the growth medium contains organic carbon sources (Chapter 3). When community A was exposed to atrazine, these three species showed the highest relative performance (deviation of observed frequency from observed relative yield, see y-Axis Fig. 4.4 A1). Although our findings show that the mixotrophic species in community A performed best under atrazine, trait-dependent complementarity stayed close to zero (Fig. 4.2). This was due to the – compared to the other species in community A – average monoculture yields of these best-performing mixotrophic species (Fig. 4.4 A2), which resulted in neither positive nor negative covariances between the species' relative performance and monoculture yields, and thus only minor trait-dependent complementarity effects. The increase of diatom EPS production at low atrazine levels thus served as a potential facilitative mechanism, which drove complementarity effects through the presence of mixotrophic species capable of utilizing these organic exudates. Since these mixotrophic species were not characterised by any above- or below average monoculture yields, the stimulation of EPS production led to an increase of trait-independent rather than trait-dependent complementarity.

In the presence of copper, both diatom communities also increased their EPS production when exposed to low levels of the metal (Fig. 4.3). Microalgae are usually among the first organisms to be affected by metal pollution due to their large surface to volume ratio (Miao et al. 2005, Quigg et al. 2006, Manimaran et al. 2012). The toxicity of heavy metals to microalgae primarily lies in the formation of reactive oxygen species, which can damage cell membranes, plastids and other intracellular structures (Masmoudi *et al.* 2013). However, microalgae can increase their metal tolerance through the excretion of extracellular polymers (Hall et al. 1979, Fisher and Frood 1980, Pistocchi et al. 1997). The excretion of EPS allows microalgae to chelate metal ions in the medium, thus reducing metal accumulation within the algal cells (Pistocchi et al. 1997). Compared to other microalgal species, diatoms are characterised by a high EPS production (De Brouwer et al. 2000). This capacity to excrete EPS under metal stress can enable diatoms to grow at copper concentrations which are up to one magnitude higher than those limiting the growth of other microalgae classes (Pistocchi et al. 1997).

In community A, most species performed better when exposed to copper in community rather than in monoculture, but this complementarity was evenly distributed amongst species (Fig. 4.4 A), and not predicted by their EPS production (Fig. 4.3 A). The positive diversity effect on the biomass yield of this diatom community under copper was thus likely caused by other mechanisms than the excretion of extracellular polymers. Such mechanisms can consist of an intracellular accumulation of metal ions in vacuoles or metal immobilization in the cell wall, selective copper exclusion from algal cells, and an increased production of amino acids protecting cell membranes from copper damage (Fernandes and Henriques 1991, Lage et al. 1994, Wu et al. 1995).

The diatoms' EPS production predicted complementarity in community B under copper stress (Fig. 4.3 B). However, this complementarity was not equally distributed amongst species, and essentially favoured *A. lineolata, Gyrosigma* sp. 1 and *N. digitoradiata*. These species had the lowest biomass yields and were least copper-tolerant in monoculture, but showed the highest relative performance when exposed to the metal in community (Fig. 4.4 B). These high relative performances did not come at the expense of other species (neutral dominance effect, Fig. 4.1 B), but were due to a trait-dependent complementarity which only benefited sensitive species with low monoculture yields, causing the increases in trait-independent complementarity to be mirrored and offset by a negative trait-dependent complementarity (Fig. 4.2 B). Diversity thus increased the biomass yield of diatom species under copper stress, but since sensitive and low-yield species benefited most from being grown in community, the net diversity effect on diatom biomass yield was not altered or even reduced at low and high copper stress respectively (Appendix III Fig. S1).

The importance of species composition in determining stress-induced changes in complementarity should be highlighted. In community A, atrazine-induced increases in complementarity and concomitantly in the net biodiversity effect were driven by the presence of mixotrophic species (*C. closterium*, *Nitzschia* sp., *N. acicularis*). These three species were not present in community B, which did not show an increase in complementarity under atrazine. In community B, the copper-induced decline in trait-dependent complementarity and concomitantly in the net biodiversity effect was driven by the low-yield species *A. lineolata, Gyrosigma* sp. 1 and *N. digitoradiata* which, except for *A. lineolata*, were not present in community A and benefited most from being exposed to the metal in community. This strong role of species composition implies that any generalisation from our results to other communities in the field should be treated very carefully, since stressor-induced changes in the net biodiversity effect will likely depend on the traits of the species present. Moreover, our experiment included only three stress levels, and the relation between EPS and complementarity was essentially driven by the high EPS

production of diatoms in the low copper and atrazine treatments. With only three stress levels and three EPS replicates per stress level, we cannot make an generalized statement on the relation of complementarity effects and EPS secretion, and additional experiments involving more stress levels would be needed to confirm the role of EPS as driver of positive biodiversity effects under stress.

Complementarity effects tend to increase over time (Fargione et al. 2007). Also, complementarity is strongest along gradients of broad environmental niche space, which allow for resource partitioning between species under natural conditions (Špaèková and Lepš 2001, Hodapp et al. 2016). Our experiments were performed over a relatively short time (one diatom growth cycle) in an artificial experimental system which limited environmental fluctuations and spatial heterogeneity, which can lead to an underestimation of complementarity effects. Also, to confirm EPS excretion as a facilitative mechanism under stress, it is recommended to quantify the extent to which extent the production of EPS reduced metal exposure of diatom cells, as well as the uptake of these organic exudates by mixotrophic species. The former could be achieved by measuring the intracellular copper concentrations in the diatoms (e.g. see Pistocchi *et al.* 1997), the latter by a colorimetric quantification of the metabolization of organic substances by diatoms (e.g. see Tuchman *et al.* 2006). Moreover, EPS production was a 'sleeping' facilitative mechanism, which was stimulated when diatom communities were exposed to chemical stress, and only predicted complementarity in diatom communities containing either stress-sensitive species, or species capable of heterotrophic growth.

We infer four conclusions from our results: first, a positive biodiversity effects cause stressed diatom communities to produce more biomass than expected from their monoculture. Second, these positive biodiversity effects are largely underpinned by complementarity effects. Third, complementarity in stressed diatom communities can potentially be driven by the release of extracellular polymers as a facilitative mechanism, leading to a better performance of species characterised by a mixotrophic growth mode or a low stress tolerance in monoculture. Fourth, a negative trait-dependent complementarity can cause the net diversity effect to decrease under stress, if low-yield species perform better when grown with different species.

Chapter 5: Selective and context-dependent effects drive chemical stressor impacts across trophic levels at the basis of marine food webs

Abstract

Human activities increasingly impact the functioning of marine food webs, but anthropogenic stressors are seldom included into ecological study designs and diet quality, as distinct from just diet quantity, has rarely been highlighted in food web studies in a stress context.

We first measured the effects of metal and pesticide stress (copper and atrazine) on the contribution of a benthic intertidal diatom community to three processes that are key to the functioning of intertidal systems: biomass production (diet quantity), lipid content (diet quality) and extracellular polymer production (sediment stabilization). We then examined if stressors affected diatom functioning by selectively targeting the species contributing most to functioning (selective stress effects) or by changing the species' functional contribution (context-dependent effects). Finally, we tested the response of the diatoms' main grazers (harpacticoid copepods) to changes in diet quality.

Diatom diet quantity was reduced by metal stress but not by low pesticide levels due to the presence of an atrazine-tolerant, mixotrophic species. Context-dependent effects of both stressors increased the diatoms' contribution to sediment stabilization by stimulating the release of extracellular polymers.

Selective effects of the pesticide reduced diatom diet quality by 60% and 75% at low and high pesticide levels respectively, by shifting diatom community structure from dominance by lipid-rich species towards dominance by atrazine-tolerant, but lipid-poor species. Context-dependent effects did not affect individual diatom lipid content at low levels of both stressors, but caused diatoms to lose 40% of their lipids at high copper stress.

Copepod lipid content was related to the quality of their diatom diet, with copepods losing half of their lipids when feeding on diatoms grown under low and high pesticide and high metal stress. Selective atrazine effects on diatom community structure affected the energy flow to their grazers at stress levels where no context-dependent effects of both stressors on diatom diet quality were detected.

Chemical stress differentially affected the contribution of marine primary producers to diet quantity, sediment stabilization and diet quality. Selective community shifts were a more potent threat for diet quality than context-dependent stress effects on primary producers. Diet quality

predicted the energy flow from marine producers to their consumers and should be considered when measuring food web functioning under anthropogenic change.

5.1. Introduction

The impact of human activities on biological communities and their contribution to ecosystem functioning has become a central topic in ecological research (Halpern et al. 2008, Cardinale et al. 2012, Gamfeldt et al. 2015). Although conservation research is framed within the context of anthropogenic change, exposure to anthropogenic stressors is rarely included into ecological study designs (McMahon et al. 2012, Steudel et al. 2012).

Stress can affect ecosystem functioning by causing biodiversity loss in terms of species richness, as well as through changes in community structure, without necessarily causing species to go extinct (Hillebrand et al. 2008, Wittebolle et al. 2009, Mensens et al. 2015). Selective stress effects on community structure (hereafter 'selective stress effects', Wittebolle et al. 2009) can reduce ecosystem functioning if stressed communities are dominated by tolerant species with a low functional contribution (Larsen et al. 2005, Mensens et al. 2015). If the functionally most important species are also most stress-tolerant, loss of functioning under stress will be limited (Radchuk et al. 2016). Moreover, functioning in stressed communities can be driven by 'context-dependent effects', i.e. changes in the species' functional contribution (Fox 2006, Fox and Harpole 2008, Tylianakis et al. 2008, Hiddink et al. 2009). Context-dependent effects can arise from direct effects of environmental drivers on the species' functional contribution (Fox and Harpole 2008, e.g. physiological stress, Schimel et al. 2007), as well as from differential species interactions under stress (e.g. changes in complementarity, Fox 2006, Fox and Harpole 2008).

The majority of experiments designed to address ecosystem functioning under anthropogenic change have focused on single trophic levels, usually primary producers (Raffaelli 2006, Cardinale et al. 2011). Stressors that alter functioning at the producer level can however have concomitant impacts on their consumers (Rohr and Crumrine 2005, McMahon et al. 2012). The trophic impacts of anthropogenic stressors are commonly examined with regard to diet quantity, which has been linked to the abundance and biomass of consumers (e.g. Kasai and Hanazato 1995, Wendt-Rasch et al. 2004, Rohr and Crumrine 2005). Far less attention has been devoted to diet quality, which considers a diet's biochemical composition and is commonly expressed as lipid content (Jodice et al. 2006, Guo et al. 2016). Nevertheless, the growth, reproduction and energy profile of consumers is strongly linked to the quality of their diet (Giles et al. 2002, Österblom et al. 2008, Taipale et al. 2013). The biochemical composition of primary producers is increasingly affected by human disturbance (Vitousek et al. 1997, Wang and Frei 2011, Guo et al. 2016, Sanpera-Calbet et al.

2016), which has made the integration of food biochemistry with traditional studies of diet quantity a key challenge for estimating food web functioning under stress (Arts and Wainmann 1999, Guo et al. 2016).

Here, we first measure the effects of metal and pesticide stress (copper and atrazine) on the structure of a benthic diatom community and its contribution to three ecosystem processes: biomass production (diet quantity), essential fatty acid (diet quality) and extracellular polymer production (sediment stabilization). Benthic diatoms are the main primary producers in many soft-sediment intertidal habitats, enhancing sediment stabilization in these systems through the production of extracellular polymeric substances (EPS, Underwood and Kromkamp 1999, Decho 2000). Diatom diet quality in terms of essential fatty acid (EFA) content plays a crucial role for trophic energy transfer (Arts et al. 2001, Taipale et al. 2013). EFAs cannot be synthesized by animals but are key determinants of the growth and energy content of aquatic consumers (Brett and Müller-Navarra 1997, von Elert 2002, Arendt et al. 2005, Litzow et al. 2006). Harpacticoid copepods are among the main consumers (grazers) of benthic diatoms and incorporate large amounts of EFAs from their algal diet, making harpacticoids pivotal for the energy transfer from primary producers to higher trophic levels (Alheit and Scheibel 1982, De Troch et al. 1998, Nanton and Castell 1998, Buffan-Dubau and Carman 2000, Andersen et al. 2005).

Next, we test if stressor-induced changes in diatom functioning (EFA and EPS production) were caused by selective or context-dependent stress effects. Context-dependent effects are measured by comparing functioning in experimental diatom communities with that in synthetic communities. Synthetic communities are computed from the species' EFA and EPS production in unstressed monocultures and reflect the same community structure observed at each stress level, thus allowing to exclude any confounding effects of community structure on diatom functioning. Selective stress effects are quantified by contrasting synthetic communities which reflect the community structure under stress and control conditions, thus excluding any confounding context-dependent effects on diatom functioning.

Last, we test if potential changes in the survival and fatty acid content of the harpacticoid copepod *Microarthridion littorale*, being the dominant grazer at the intertidal study site, are related to atrazine and copper effects on the quality of its diatom diet. For instance, whether potential changes in grazer fatty acid content are linked to context-dependent stress effects on the quality of its diatom diet, or to changes in community structure leading to dominance of diatoms with a low or high contribution to energy flow in intertidal systems.

5.2. Methods

Experimental organisms & culture conditions. The harpacticoid copepod *Microarthridion littorale* (family Tachidiidae) was collected from intertidal mud at the Paulina intertidal flat (SW Netherlands, 51° 21'N, 3°43'E), where it represented the dominant grazer (~ 90% of all harpacticoid individuals). *M. littorale* specimens were extracted alive from the sediment using a mixed technique of sediment decantation and extraction via white light attraction. Adult specimens were randomly collected with a glass pasteur pipette using a Wild M5 binocular. Copepods were washed 3 times over a 38 µm sieve and placed in glass jars with filtered and autoclaved natural seawater overnight in order to empty their intestines prior to the start of the experiment.

The diatom community was composed of six species representing the most abundant genera observed at the sampling site (Addendum IV Table S1). For this experiment, all species were obtained from the culture collection of the Protistology and Aquatic Ecology Research Group (UGent) (http://bccm.belspo.be). Prior to the set-up of the experiments, the diatoms were grown in tissue bottles (Greiner BioOne, CELLSTAR® TC, 175 cm^2 growth surface) during 10 days in a climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons $m^{-2}s^{-1}$, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients (Guillard 1975).

Diatom experiments. The experimental diatom communities were exposed in five treatments to 0, 200 (hereafter 'low') and 500 (hereafter 'high') µg/l atrazine and copper respectively, representing levels of atrazine and copper stress resulting on average in a 50% and 90% reduction in growth of the diatom species (Chapter 3). Atrazine treatments were prepared from a stock solution obtained by dissolving 50 mg technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure, Sigma-Aldrich Chemie Gmbh, Munich, Germany) in 10 ml acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.01% acetone in the treatments. An acetone control treatment of 0.01% acetone was included and compared to an acetone-free control to test for carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a $Cu[II]Cl_2$ solution, analytical grade; VWR International) was spiked directly into the culture medium before exposure of the diatoms. F/2 culture medium for the copper experiments was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The obtained atrazine and copper concentrations that were finally applied in the experiment are listed in Addendum IV Table S2. Additionally, the six diatom species were assembled in monoculture under control conditions, to quantify each species' biomass, EFA and EPS production in the absence of the stressors (Addendum IV Table S1). All treatments were run in tissue culture flasks (Greiner BioOne, CELLSTAR® TC, 175 cm^2

growth surface), with nine replicates per treatment (three replicates in the monoculture treatments). Each microcosm was inoculated with a total cell density of approximately 5000 diatom cells/ml (500000 cells in 100 ml medium per culture flask, between 800-850 cells/ml per species in the diatom communities) from exponentially growing cultures, and incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons $m^{-2}s^{-1}$. Culture medium was refreshed after eight and 15 days, diatom biofilms as food for the copepod experiment as well as for the EPS and EFA analyses (see further) were harvested after 15 days (late exponential growth phase) and experiments were terminated after 25 days.

Diatom biofilms in three replicates of 100 ml cell suspension per treatment were harvested as food for the corresponding treatments in the copepod experiment, and purified from copper and atrazine. In the purification process, the diatom cells were resuspended and placed in 50 ml centrifuge tubes (2 tubes per replicate). All samples were then centrifuged at a speed of 50*g* for 10 minutes. Supernatant fluid was removed and replaced with f/2 culture medium (25 ml total volume) and centrifuged again for 10 minutes. After the second centrifuge process, supernatant fluid was removed again, and 5 ml of concentrated diatom pellet per replicate were transferred to nine Eppendorf microtubes (0.56 ml pellet per microtube), freeze-dried and preserved at -80 °C. The individual microtubes contained the food aliquot for each day of the respective treatments in the copepod experiment (see further).

Copepod experiment. We tested the effect of diatom diet quality by offering *M. littorale* diatom diets of equal biomass under unstressed conditions. The copepod experiment consisted of five treatments, each with three replicates of 100 *M. littorale* copepods (a natural mix of adult males and (gravid) females), that were fed for 10 d an equal biomass of diatoms grown under unstressed conditions and low and high atrazine and copper stress, respectively. The experiment was conducted in glass jars containing 100 ml of filtered and autoclaved seawater in a climate room at 15±1 °C with a 12:12 h light:dark cycle and 40 to 50 µmol photons m^{-2} s^{-1}. To ensure a constant food supply, each treatment was inoculated with a concentrated diatom pellet of 0.23 mm^3 biovolume every day, and unconsumed diatoms were removed from the bottom of the jars. Over the whole duration of the experiment, a total diatom biovolume of 2.05 mm^3 was applied per experimental unit, corresponding to 3 to 5 x 10^6 diatom cells per treatment. At the end of each day, there was no food depletion in any of the treatments. Based on our previous experiments (De Troch et al. 2005, 2007), the provided quantity of diatoms can be considered as above the feeding saturation level. Copepod mortality was determined at the end of the experiment, and surviving (85-100%) individuals from each experimental unit were washed in natural seawater to remove food particles, left 12h to empty their gut, and stored at – 80 °C for further fatty acid analysis.

Biomass, EPS and EFA analyses. Diatom biomass was quantified as biovolume after 0, 2, 5, 10, 15, 20 and 25 days of incubation. Biovolume was calculated from cell densities, linear dimensions (measured digitally using ImageJ, Schneider et al. 2012) and formulas representing the closest approximation of geometric shape for each genus (Hillebrand et al. 1999, Addendum IV Table S1). Cell densities (in cells/ml) were determined by digitally counting the cells (ImageJ cell counting software) in photographs obtained by magnifying and photographing (x100) an area of 0.66 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11). All further analyses use the biomass on day 15.

Extracellular polymeric substances (EPS) secreted by the diatom communities were measured in three replicates of 10 ml diatom suspension which were collected after 15 days of incubation. EPS concentrations were analysed by spectrophotometry according to a modified version of the phenol/H_2SO_4 assay (Dubois et al. 1956) as described in Mensens *et al.* (2015).

Essential fatty acids (EFAs) as marker of diatom diet quality were quantified as the content of eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). Three replicates of 8 ml suspended diatom culture per treatment of the diatom communities and monocultures were collected after 15 days. The samples were centrifuged for 10 minutes at 10 °C and a speed of 50*g*. After undergoing the same purification process as the diatom food samples for the grazer experiment, pellets were resuspended and 1.5 ml of concentrated diatom suspension per replicate were placed in a glass vial and stored at -80 °C for fatty acid analysis.

Copepod and diatom EFA content was measured through hydrolysis of total lipid extracts and methylation to FA methyl esters (FAME), followed by the analysis of the obtained FAME using a gaschromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973) according to the protocol described in De Troch *et al.* (2012) for copepods and Mensens *et al.* (2015) for diatoms. The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 ng/ml). All EPS and EFA concentrations were standardized to diatom biomass or the number of copepod individuals for diatoms and copepods, respectively.

Data analysis. An analysis of the diatom community structure among the treatments (control, low and high atrazine and copper) was conducted with a non-metric multidimensional scaling method based on Bray-Curtis similarity. A one-way analysis of similarity (ANOSIM) was used to test for significant biomass differences between the treatments. Subsequently, percentages of similarity (SIMPER) were calculated to determine the main species contributing to any differences in community structure.

Differences in diatom biomass, EFA and EPS production among the treatments were tested with a generalized least squares model, with biomass, EFA and EPS as response variables and treatment type as categorical predictor (Equation 1)

$$Y \sim \beta_T \cdot T \quad [1]$$

where Y is the response variable (biomass, EFA or EPS), T is the treatment type (control, low and high atrazine and copper), and the beta coefficient β_T is the slope measuring the effect of the treatment type on biomass, EFA or EPS production. Biomass, EFA and EPS production among treatments were compared with pairwise Tukey's tests correcting p-values for multiple comparisons by the single-step method.

Next, we tested if potential changes in diatom EFA and EPS production were due to selective or context-dependent effects of atrazine and copper. Selective and context-dependent effects were quantified by comparing the EFA and EPS production in experimental and synthetic diatom communities. The synthetic communities have the same community structure as observed in the corresponding treatments of the experimental communities, but are computed from the species' EFA and EPS production in unstressed monocultures (Equation 2). The synthetic communities thus reflect the EFA and EPS production expected at the same community structure as induced by copper and atrazine, however without any stress exposure or species interactions.

$$Y_{SYN,j} = \frac{\sum_{i=1}^{N} M_{i,j=0} \times B_{i,j}}{B_{T,j}} \quad [2]$$

$Y_{SYN,j}$ is the EFA or EPS production in synthetic communities of the same structure as the experimental communities at the atrazine or copper concentration *j*. $M_{i,j=0}$ is the mean EFA or EPS production per unit biomass of species *i* in monoculture under unstressed conditions (*j*=0). $B_{i,j}$ is the biomass of species *i* observed in the experimental community at the stress level *j*. $B_{T,j}$ is the total biomass observed in the experimental community at the stress level *j*.

Selective and context-dependent effects of both stressors were analysed with a generalized least squares model (Equation 3) and pairwise comparisons of the EFA and EPS production in experimental and synthetic diatom communities.

$$Y \sim \beta_T \cdot T \quad [3]$$

where Y is the diatom EFA or EPS production per unit biomass, T is the treatment type (control, low and high atrazine and copper in the experimental and synthetic diatom communities), and the beta coefficient β_T is the slope measuring the effect of the treatment type on EFA or EPS

production. Pairwise comparisons were performed with a Tukey's test correcting p-values for multiple comparisons by the single-step method.

Context-dependent effects occur when synthetic and experimental communities within the same treatment differ in their EFA or EPS production. Since both community types have the same structure, any differences in EFA or EPS between the two community types result from direct stress effects or species interactions in the experimental community. Consequently, any differences between experimental and synthetic communities of the same treatment point at context-dependent effects.

Selective stress effects occur when synthetic communities reflecting the control community structure differ in their EFA or EPS production from synthetic communities reflecting the community structure under stress. Since synthetic communities are computed from unstressed monocultures, any differences in EFA or EPS between synthetic communities are related to differences in community structure rather than direct stress effects or species interactions. Consequently, any differences among synthetic communities are linked to selective rather than context-dependent effects. Addendum IV Fig. S1 provides a scheme visualizing the quantification of context-dependent and selective stress effects.

The response of copepod fatty acid content to stressor-induced alterations in the quality and community structure of their diatom diet was analysed with generalized least squares models, with copepod fatty acid content as response variable and diatom diet quality and community structure as predictors (Equation 4). Diatom diet quality was quantified as EFA production, diatom community structure as the Bray-Curtis percent similarity to the average community structure in controls (Equation 5). Models were fitted separately for copepods feeding on atrazine- and copper-exposed diatoms respectively, to test if the effects of either stressor on copepod fatty acid content can be predicted from changes in diatom diet quality or community structure.

$$E_C \sim a + b \cdot E_D + c \cdot C_D \qquad [4]$$

E_C is the copepod fatty acid content (EFA content per copepod individual), E_D is diatom diet quality (EFA production per unit biomass), C_D is diatom community structure, a is the intercept, b and c represent the slopes, i.e. the relation of copepod EFA content to diatom diet quality and diatom community structure. If E_D and C_D were correlated (correlation factor > 0.5), models were fitted separately for both predictors.

$$C_D = 100 \cdot \{1 - \Sigma |B_{i,j} - \mu B_{i,j=0}| / \Sigma(B_{i,j} + \mu B_{i,j=0})\} \quad [5]$$

$B_{i,j}$ is the biomass of species *i* at the atrazine or copper concentration *j* and $\mu B_{i,j=0}$ is the mean biomass of species *i* in the control (*j*=0).

For all least squares model fits, normality and homogeneity of model residuals were inspected by evaluation of quantile-quantile plots and Shapiro-Wilk's test, and by Levene's test and plotting residuals versus explanatory variables respectively. Untransformed data did not violate normality (Shapiro-Wilk's test, $\alpha > 0.1$). If indications of deviations from normality were detected ($0.1 < \alpha < 0.15$), an optimal Box-Cox transformation (Box and Cox 1964, Venables and Ripley 2002) was applied to maximize normality of model residuals. If homogeneity was violated, the model was refitted using an exponential variance structure allowing residuals to change with the continuous predictor X (weights = varExp(form ~ 1 | X) or allowing different variances according to the categorical predictor P (weights = varIdent(form ~ 1 | P). By means of likelihood ratio testing, the significance of these structures was tested ($\alpha = 0.05$).

Multivariate, ANOSIM and SIMPER analyses of diatom community structure were performed using Primer 6 software (Clarke and Gorley 2006). All other calculations were done in R 3.0.1. using RStudio (R Development Core Team 2016). The package nlme (Pinheiro et al. 2016) was used for the fitting of generalized least squares models and optional variance structures. Optimal Box-Cox transformations were performed using MASS (Venables and Ripley 2002). Pairwise Tukey's tests on the fitted models were performed with the package multcomp (Hothorn et al. 2008), using the general linear hypothesis test (glht) function, correcting p-values for multiple comparisons by the single-step method (default procedure in multcomp).

5.3. Results

Diatom community structure. The structure of diatom communities under atrazine differed from the structure of communities grown under control conditions and copper exposure (see non-metric multidimensional scaling in Addendum IV Fig. S2). The ANOSIM confirmed the significance of treatment type for community structure (global R = 0.833, p=0.001). The community structure at both copper levels resembled the community structure observed under control conditions (14% and 18% dissimilarity respectively), with *N. acicularis* and *N. arenaria* contributing the most biomass in both types of communities (Fig. 5.1, Addendum IV Table S3). In contrast, both atrazine levels induced a change in community structure (70% and 76% dissimilarity from the control) due to an increase in biomass of *C. closterium*, which compared to the control showed a 6- and 12-fold increase in biomass in the high and low atrazine treatments, respectively. This resulted in a

dominance by *C. closterium* in the atrazine-exposed communities, as it contributed more than 70% of the total biomass at both atrazine levels (Fig. 5.1, Addendum IV Table S3). Within the control, copper and atrazine treatments, the community structure of diatom communities showed little variance (within-treatment similarities between 84% and 92%, Addendum IV Table S4).

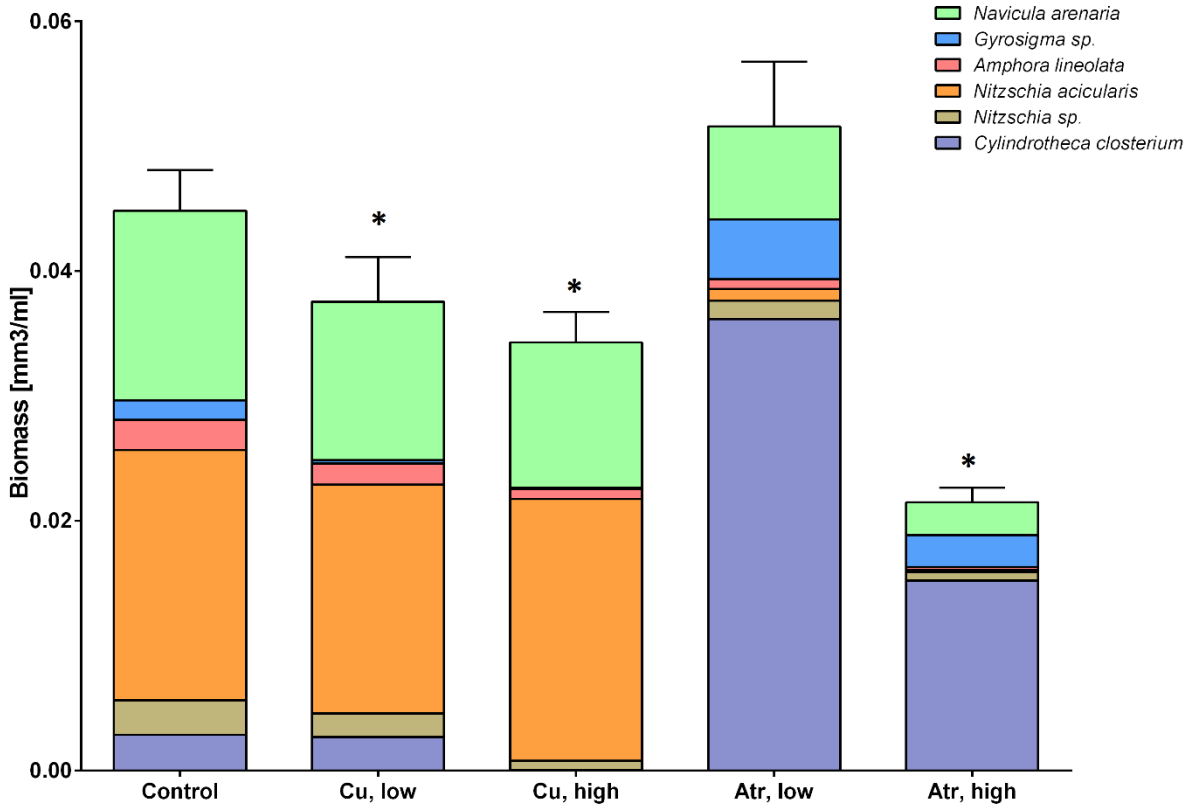


Fig. 5.1: Total biomass production per treatment and biomass of the component species for diatom communities grown in control, low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') treatments. Significant differences in biomass production from the control are indicated with asterisks (*).

Diatom biomass, EPS and EFA production. Diatom biomass, EPS and EFA production changed depending on treatment type (all $p<0.0001$, Fig. 5.1, Fig. 5.2). The post-hoc analyses showed that diatom biomass was reduced at both low and high copper as well as at high but not at low atrazine concentrations (Fig. 5.1, Table 5.1). Diatom EPS production increased at low atrazine and copper stress, with diatom communities producing twice as much EPS than under control conditions, but was not affected at high levels of both stressors (Fig. 5.2A grey bars, Table 5.1). Diatom EFA production was reduced at both levels of atrazine, but only at high copper stress (Fig. 5.2B grey bars, Table 5.1). High copper stress reduced the EFA production of diatom communities by 40%, low and high atrazine stress by 60% and 75%, respectively (Fig. 5.2B grey bars).

Process	Treatment	Treatment	Est	lwr	upr	Z	P
Biomass	Control	Atr, low	-6.756	-9.18	-4.33	2.78	0.059
		Atr, high	23.327	20.90	25.75	9.61	**<0.001**
		Cu, low	7.273	4.85	9.70	-3.00	**0.036**
		Cu, high	10.533	8.11	12.96	4.34	**<0.001**
	Atr, low	Atr, high	30.083	27.66	32.51	12.39	**<0.001**
	Cu, low	Cu, high	3.260	0.83	5.69	1.34	0.666
EPS	Control	Atr, low	-0.251	-0.44	-0.06	-4.03	**<0.001**
		Atr, high	0.066	-0.13	0.26	1.06	0.984
		Cu, low	-0.290	-0.48	-0.10	-4.66	**<0.001**
		Cu, high	0.016	-0.18	0.21	0.25	1.000
	Atr, low	Atr, high	0.317	0.13	0.51	5.09	**<0.001**
	Cu, low	Cu, high	0.305	0.11	0.50	4.91	**<0.001**
EFA	Control	Atr, low	2.143	1.30	2.99	8.00	**<0.001**
		Atr, high	3.191	2.34	4.04	12.08	**<0.001**
		Cu, low	-0.046	-0.89	0.80	-0.17	1.000
		Cu, high	1.372	0.52	2.22	5.12	**<0.001**
	Atr, low	Atr, high	0.317	0.13	0.51	5.72	**<0.001**
	Cu, low	Cu, high	0.305	0.11	0.50	4.91	**<0.001**

Table 5.1: Pairwise comparisons of biomass, EFA and EPS production in diatom communities as estimated by generalised least squares model fits. 'Process' indicates to which response variable models were fitted (biomass, EFA or EPS production). 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicates which treatments are compared. 'Est' indicates the difference in biomass (in 10^3 mm^3/ml), EFA (in $\mu g/mm^3$) and EPS (in mg/mm^3) production between the compared treatments as estimated by generalized least squares models fitted to untransformed biomass and Box-Cox transformed EFA and EPS data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

Selective and context-dependent stress effects on diatom EPS and EFA production. The EPS and EFA production in the experimental and synthetic diatom communities changed depending on treatment type (both $p<0.0001$). Pairwise comparisons of the EPS production in the experimental and synthetic communities showed no difference among the two community types in the control and the high copper and atrazine treatments (Fig. 5.2A, Table 5.2). At low levels of both stressors however, the EPS production in experimental communities was double of that expected in unstressed synthetic communities of the same structure (Fig. 5.2A, Table 5.2). Within the synthetic communities there was no difference in EPS among any of the treatments (Fig. 5.2A, Table 5.2).

Pairwise comparisons of the EFA production in experimental and synthetic communities did not show differences among the two community types in the control and low copper treatments (Fig. 5.2B, Table 5.2). At high copper stress, the EFA production in the experimental community was lower than in the corresponding synthetic community (Fig. 5.2B, Table 5.2). The EFA loss induced by high copper stress in experimental communities was thus not reflected in unstressed synthetic

communities of the same structure, whose EFA production did not differ from the control (Fig. 5.2B, Table 5.2).

In the atrazine treatments, both the experimental and synthetic communities had a lower EFA production than the control (Fig. 5.2B, Table 5.2). Within each atrazine treatment, the EFA production of experimental and synthetic communities did not differ (Fig. 5.2B, Table 5.2). The EFA loss induced by atrazine in the experimental communities was thus reflected by the synthetic communities, which mimicked the community structure under atrazine without actual exposure to the herbicide (Fig. 5.2B, Table 5.2).

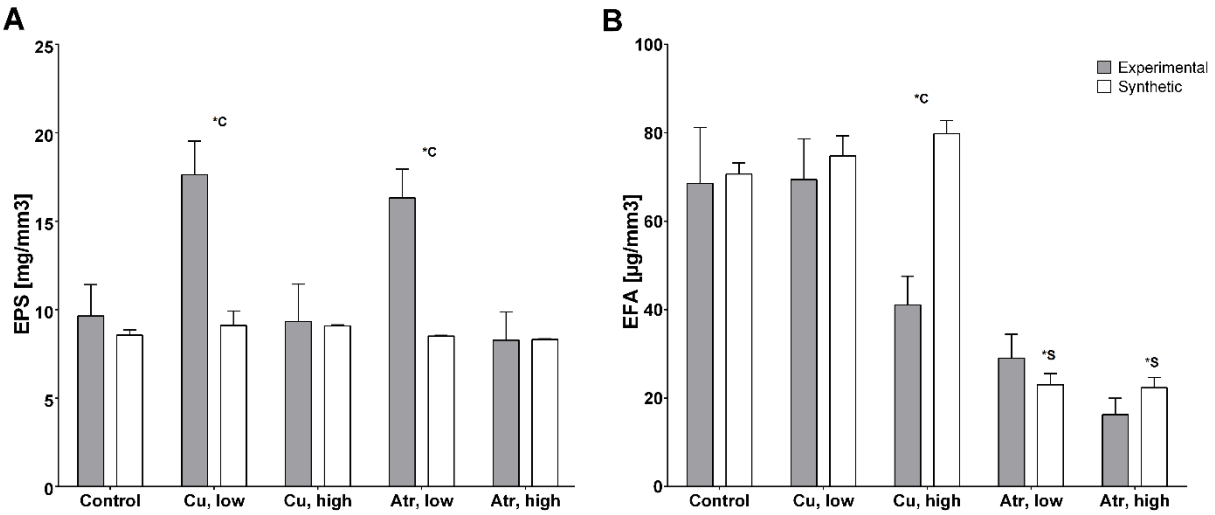


Fig. 5.2: Atrazine and copper effects on diatom sediment stabilization (EPS production, panel A) and diet quality (EFA production, panel B). Experimental communities were grown in control, low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') treatments. Synthetic communities were computed from unstressed monocultures in the same community structure observed at each stress level. Significant differences in EFA or EPS production indicating selective and context-dependent stress effects are indicated with asterisks (*). *C indicates pairwise differences among stressed and synthetic communities of the same structure (context-dependent stress effects). *S indicates pairwise differences among control and stress treatments within the synthetic communities (selective stress effects).

Process	Effect	Com	Treatment	Com	Treatment	Est	lwr	upr	Z	P
EPS	SE	Syn	Control	Syn	Atr, low	0.002	-0.05	0.05	0.15	1.000
					Atr, high	0.012	-0.04	0.05	0.78	0.998
					Cu, low	-0.026	-0.07	0.02	-1.68	0.765
					Cu, high	-0.026	-0.07	0.02	-1.65	0.779
	CD	Exp	Control	Syn	Control	0.047	-0.09	0.18	-1.05	0.984
			Atr, low		Atr, low	0.301	-0.16	0.44	6.64	**<0.001**
			Atr, high		Atr, high	-0.006	-0.15	0.13	-0.13	1.000
			Cu, low		Cu, low	0.311	0.17	0.45	6.86	**<0.001**
			Cu, high		Cu, high	0.006	-0.13	0.15	0.14	1.000
EFA	SE	Syn	Control	Syn	Atr, low	2.695	1.85	3.54	10.06	**<0.001**
					Atr, high	2.746	1.90	3.60	10.25	**<0.001**
					Cu, low	-0.173	-1.02	0.67	-0.65	0.999
					Cu, high	-0.381	-1.23	0.47	-1.42	0.921
	CD	Exp	Control	Syn	Control	-0.110	-0.96	0.74	0.41	1.000
			Atr, low		Atr, low	0.442	-0.41	1.29	1.65	0.823
			Atr, high		Atr, high	-0.554	-1.40	0.29	-2.07	0.550
			Cu, low		Cu, low	-0.237	-1.08	0.61	0.89	0.997
			Cu, high		Cu, high	-1.863	-2.71	-1.02	-6.95	**<0.001**

Table 5.2: Pairwise comparisons of EFA and EPS production in treatments of experimental and synthetic diatom communities as estimated by generalised least squares model fits. 'Process' indicates to which response variable models were fitted (EFA or EPS production). 'Effect' indicates which type of stress is analyzed: Selective stress effects ('SE': comparison of synthetic communities reflecting the community structure under control and stress conditions), Context-dependent stress effects ('CD': comparison of stressed and synthetic communities of the same community structure). 'Com' ('Exp': Experimental and 'Syn': Synthetic) and 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicate which communities and treatments are compared. 'Est' indicates the difference in EFA and EPS production between the compared treatments as estimated by generalized least squares models fitted to Box-Cox transformed EFA and EPS data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

Diet quality effect on copepods. The EFA content of *M. littorale* was related to the stressor-induced changes in diatom diet quality (both stressors) and diatom community structure (atrazine only, Fig. 5.3, Table 5.3). Copepods maintained their control EFA content when feeding on diatoms from the low copper treatment, but lost half of their EFAs when feeding on diatoms grown under high copper stress (Fig. 5.3). This resulted in a positive correlation of copepod EFA content and diatom EFA content (Fig. 5.3, Table 5.3). When offered diatoms from the low and high atrazine treatments, copepods also lost half of their EFA content, which was predicted not only by the diatoms' EFA content, but also by the changes in diatom community structure (Fig. 5.3, Table 5.3). Addendum IV Fig. S3 shows the EFA content per diatom biomass and per copepod individual, as well as the relative proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In diatoms, the essential fatty acids consisted mainly of EPA, whereas DHA was the main component in copepods (EPA:DHA ratio 4 to 6 in diatoms, 0.3 to 0.6 in copepods, Addendum IV Fig. S3). The copepod survival rate in the experimental units was between 85-100%. The EFA

content of copepods feeding on diatoms from control conditions (65.3 ± 6.1 ng copepod^{-1}) did not differ significantly from copepods at the start of the experiment (i.e. animals collected in the field, 68.8 ± 11.8 ng copepod^{-1}, t-test, p=0.99, Addendum IV Fig. S3).

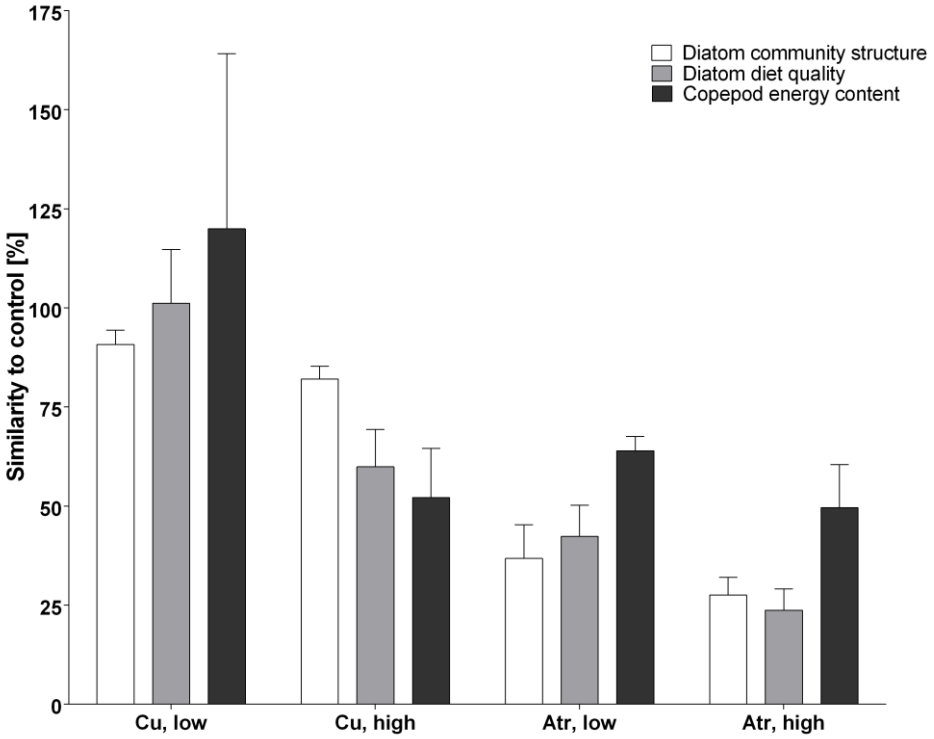


Fig. 5.3: Community structure and diet quality (EFA content) of diatoms exposed to stress, and EFA content of *Microarthridion littorale* after 10d of feeding on the respective diets, visualised as percent similarity to the corresponding control. The similarity of diatom community structure is calculated as the Bray-Curtis similarity to the control mean (see methods section). The similarity of diatom and copepod EFA content is calculated as fraction percentage of the EFA concentration in treatment *i* and the mean (μ) EFA concentration in corresponding control c: ($EFA_i/\mu EFA_C$)x100. Diatom communities were exposed to low (200 μg/L) and high (500 μg/L) atrazine ('Atr') and copper ('Cu') concentrations, copepods were not exposed to any of the stressors.

Stressor	Quality	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Atrazine	EFA	1	0.059	0.008	7.38	**0.0002**	28.46	-11.23	Yes	0.69	0.41
		2	0.060	0.007	9.06	**<0.0001**	29.77	-10.89	Yes		
	Comp	1	0.045	0.008	5.88	**0.0006**	31.69	-12.85	Yes	0.07	0.79
		2	0.045	0.008	5.64	**0.0008**	33.62	-12.81	Yes		
Copper	EFA	1	0.137	0.021	6.57	**0.0003**	35.21	-14.61	Yes	3.64	0.06
		2	0.133	0.014	9.17	**<0.0001**	33.57	-12.79	Yes		
	Comp	1	0.204	0.126	1.62	0.1486	44.82	-19.41	No	0.75	0.39
		2	0.071	0.150	0.48	0.6494	46.07	-19.03	Yes		

Table 5.3: Results of generalized least squared models predicting copepod EFA content from diatom community structure and diet quality (EFA production). 'Stressor' denotes which diet the copepods were offered: diatom communities exposed to copper or atrazine. 'Quality' indicates the predictor: diet quality in terms of diatom community composition or EFA production. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) variance structure. 'Slope' indicates the relation between predictors and copepod EFA content, i.e. the effect of diatom diet quality and community structure on copepod EFA content. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. ; 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood . 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change with the predictor. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

5.4. Discussion

The outcome of our experiments demonstrates that chemical stress can stimulate the contribution of marine primary producers to sediment stabilization while reducing diet quantity and diet quality. Selective and context-dependent stress effects on diet quality were furthermore found to lead to concomitant knock-on effects at the consumer level.

Biomass production is the most widespread functional endpoint in research investigating ecosystem functioning under anthropogenic change, and producer biomass (diet quantity) has been the emphasis of most trophic experiments (Arts and Wainmann 1999, Balvanera et al. 2006, Cardinale et al. 2011). The loss of diatom biomass induced by copper corresponds to previous findings on the toxicity of copper to marine diatoms (Stauber and Florence 1989, Cid et al. 1995, Pistocchi et al. 1997, Masmoudi et al. 2013). Surprisingly, diatom biomass was less affected by atrazine, although atrazine has been shown to reduce diatom biomass at concentrations lower than those used in our study (Bester et al. 1995, DeLorenzo et al. 1999, 2001, Magnusson et al. 2008). The capacity of diatom communities to maintain their biomass under atrazine exposure was related to a change in community composition, with *C. closterium* increasing its biomass in

the presence of atrazine, and dominating all communities in the atrazine treatments. Atrazine blocks the electron transport chain of Photosystem II (PSII, Dorigo and Leboulanger 2001). Some diatom species can however reduce their dependency on photosynthesis and thus their sensitivity to PSII inhibitors by mixotrophic growth (i.e. the uptake of organic substrates, Debenest et al. 2009; Larras et al. 2014). *C. closterium* is capable of mixotrophic growth, which reduces its sensitivity to herbicide stress (Vanelslander et al. 2009, Chapter 3). The presence of a mixotrophic, atrazine-tolerant species thus caused the changes in community composition and underpinned the limited loss of biomass under atrazine.

Neither atrazine nor copper reduced the diatoms' contribution to sediment stabilization. On the contrary, EPS production doubled at low levels of both stressors. In our low atrazine and copper treatments, diatom EPS production was also double than expected in synthetic communities of the same community structure. The increase in EPS production thus resulted from context-dependent effects rather than from changes in community structure (Fig. 5.3 A). The secretion of EPS by microphytobenthos does not only play a vital role for ecosystem functioning through the stabilization of sediment surfaces, but also represents a physiological response of benthic diatoms to various abiotic stressors (Pistocchi et al. 1997, Decho 2000, Staats et al. 2000a, Underwood and Paterson 2003, Levy et al. 2007). At sublethal stress levels, context-dependent stress effects are thus unlikely to cause a reduction in the diatom's contribution to sediment stabilization. Selective stress can reduce diatom EPS production, by causing dominance by tolerant species producing little amounts of EPS (Mensens et al. 2015). The dominant species in our atrazine, copper and control communities however showed little difference in their EPS output, which resulted in an absence of selective stress effects on EPS production.

Selective atrazine stress determined the diet quality of diatom communities, which lost 60% and 75% of their essential fatty acids at low and high levels of the pesticide. The same extent of energy loss was observed in synthetic communities which reflected the community structure under atrazine without actual exposure to the pesticide (Fig. 5.2 B). Conversely, the diet quality in atrazine-exposed experimental communities and unexposed synthetic communities did not differ. The energy loss under atrazine was thus caused by selective changes in community structure rather than by context-dependent atrazine effects on diatom diet quality. This selective atrazine stress was underpinned by the dominant species *C. closterium*, which produced eight and three times less EFAs than the species contributing the most biomass under control conditions (*N. acicularis* and *N. arenaria* respectively, see fatty acids per species in Addendum IV Table S1). In the presence of copper, communities were dominated by the same lipid-rich species as in the control, and no such selective effects on diet quality were observed. The loss of diatom diet quality at high copper levels was caused by context-dependent rather than selective copper effects, which

was reflected in the lower diet quality in experimental compared to synthetic communities (Fig. 5.2 B). Context-dependent effects also likely caused the further loss of EFAs at high compared to low atrazine stress, since community structure at the two herbicide levels did not differ. This loss of diet quality at high levels of both stressors could be due to physiological stress effects such as an alteration of photosynthesis and thus of the carbon supply for fatty acid synthesis, an inhibition of the enzymes involved in lipid biosynthesis or an increase in the degree of fatty acid saturation, which are all reported to reduce the EFA production of microalgae under metal and pesticide stress (Böger et al. 2000, Guschina and Harwood 2006, Chia et al. 2013a).

In our study system, selective stress however proved to be the main driver of microalgal diet quality. Selective atrazine stress caused a more important loss of diatom diet quality than the context-dependent effects of both stressors, at chemical concentrations where no context-dependent effects on individual EFA content were recorded. The high and low copper and atrazine concentrations used in our experiments are respectively far above field concentrations, or have only been recorded in extreme pollution events or at chronically contaminated sites (Millward and Grant 2000, Graymore et al. 2001, Pennington et al. 2001, Lockert et al. 2006). Apart from scenarios of severe chemical pollution, pesticide and metal stress are thus unlikely to reduce the energy flow in intertidal systems by inhibiting algal EFA synthesis. Conversely, chemical pollution can cause shifts in algae community structure at stress levels lower than those used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010). The impact of such selective chemical stress on ecosystem functioning will largely depend on the functional importance of the most stress-tolerant species (Larsen et al. 2005, Mensens et al. 2015, Radchuk et al. 2016). Not only pollution, but also long-term changes in environmental variables are causing considerable shifts in microalgae community structure (Pomati et al. 2012, Litchman et al. 2015). Due to the pronounced differences in lipid profiles within and among algal classes (Dunstan et al. 1993, Taipale et al. 2013, Guo et al. 2016), these changes in community structure rather than context-dependent changes in algal diet quality could represent the potentially stronger driver of trophic energy flow under anthropogenic change.

The essential fatty acid content of the copepod *M. littorale* closely tracked that of its diets. Selective and context-dependent stress effects on diatom diet quality resulted in a concomitant loss in the EFA content of their main copepod grazer, confirming algal EFAs as a key component of diet quality which is directly linked to trophic energy transfer. The DHA:EPA ratio of *M. littorale* was higher than in the diatom communities, which corresponds to previous findings on the relative concentrations of both essential fatty acids in copepods and their algal diets (De Troch et al. 2012a, Arndt and Sommer 2014). Diatoms are characterised by a high EPA content (Taipale et al. 2013, Guo et al. 2016), but DHA appears to be the most important fatty acid for copepods (Taipale et al.

2013). Planktonic primary consumers such as cladocerans or calanoid copepods directly depend on the DHA taken up from their diet (Bell et al. 2007, Bell and Tocher 2009, De Troch et al. 2012a), but several harpacticoid copepod species are able to bioconvert EPA to the longer chain DHA, a capacity which has notably been demonstrated in *M. littorale* (De Troch et al. 2012a). While the total EFA content of *M. littorale* reflected that of its different diatom diets, this capacity to convert EPA to DHA likely enabled *M. littorale* to maintain high relative levels of DHA.

In this study we used a novel design which allowed to isolate functional changes resulting from selective changes in community structure, by calculating synthetic communities from unstressed monocultures. This design however eliminated diversity effects such as species interactions, which can drive the functional contribution of communities along environmental gradients (Tylianakis et al. 2008, Maestre et al. 2010, Chapter 4). It should thus be noted that our experiments highlight potential functional impacts of selective stress, but do not allow to quantify diversity effects on diatom functioning. Our results also have to be treated carefully due to the limited number of stress levels and replicates. To obtain a more robust correlation of the consumer response to stressor-induced changes in diet quality, models should be fitted to gradients containing more than three concentrations per stressor and more than three replicates per treatment. Also, offering *M. littorale* preserved rather than live diatom food might have influenced food uptake. Freeze-drying does not alter the biochemical composition within diatoms cells, but modifies the exterior of diatom cells through the loss of exudates (e.g. EPS) or bacteria associated to the diatom frustule, which can affect the ingestion of diatoms by harpacticoid copepods (Cnudde et al. 2011). Feeding *M. littorale* live diatom cultures under unstressed conditions would however have resulted in a dissimilar diatom community structure than that induced by the stressors: atrazine and copper did not eliminate any of the diatom species, but caused alterations of community evenness, which could not be maintained when diatoms were grown in the absence of the stressors.

Since their EFA content ranks among the highest of all algae classes, diatoms are regarded as high-quality food source and crucial link for the energy flow at the basis of aquatic food webs (Brett and Müller-Navarra 1997, Taipale et al. 2013, Guo et al. 2016). Here, diatom diet quality responded more sensitively to chemical stress than the diatom's contribution to habitat structure (sediment stabilization) and diet quantity. The selective and context-dependent stress effects on diatom diet quality were caused by the large interspecific differences in EFA content and the loss of diatom EFA content under stress. Indeed, the diet quality of benthic diatoms shows more interspecific variation and is more affected by chemical pollutants than their contribution to diet quantity or sediment stabilization (Mensens et al. 2015, Chapter 3).

Losses in diet quality occurred at copper concentrations which were close to the LC50 value of *M. littorale* (Addendum V). Next to indirect effects on copepod EFA content through changes in diet quality, high metal stress can thus be expected to reduce copepod abundance through direct toxic effects. Conversely, atrazine changes microalgal community structure at concentrations lower than the 200 µg/L used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010), whereas direct acute effects of atrazine on copepods and other marine invertebrates mostly occur at >1 mg/L atrazine (Hall et al. 1995, Bejarano and Chandler 2003). Atrazine could thus first affect copepods indirectly through selective changes in the structure of their diatom diet rather than through direct effects on the copepods themselves. The loss of diet quality did not result in increased harpacticoid mortality. Low diet quality rarely causes acute copepod mortality, but reduces copepod EFA content and, on a longer term, growth rate and reproduction (Müller-Navarra 1995, Müller-Navarra et al. 2000, Arendt et al. 2005, Gonçalves et al. 2011). The EFA content of copepods is crucial for their main consumers, especially larval fish whose development can depend on the EFAs taken up from their copepod prey (Sargent et al. 1995, Payne et al. 1998). Whilst in this study losses in diet quality did not eliminate consumers, low diet quality could thus reduce the energy transfer at the plant-animal interface, which is a key limiting factor for the functioning of marine ecosystems (Brett and Müller-Navarra 1997, De Troch et al. 2012b). Nonetheless, the importance of algal diet quality, as distinct from just diet quantity, is rarely highlighted in research on food web functioning (Guo et al. 2016). Due to its sensitive response to selective stress, algal diet quality in terms of EFA production and community structure provides a powerful approach to integrate our understanding of coastal ecosystem functioning under anthropogenic change.

In conclusion we infer three statements from our results. First, chemical stress differentially affects the contribution of marine primary producers to diet quantity, sediment stabilization and diet quality, with diet quality being most sensitive in this study. Second, the loss of diet quality is underpinned by selective and context-dependent stress effects. In this study, selective stress caused a more important loss of diatom diet quality than context-dependent stress effects, and thus represented the main risk for energy flow to their copepod consumers. Third, changes in diatom diet quality can affect copepod energy content. The integration of diet quality within traditional studies of diet quantity is recommended to assess energy flow in marine food webs under anthropogenic change.

6. General discussion

Since the first BEF experiments were conducted in the 1990s (Naeem et al. 1994, Tilman and Downing 1994, Tilman et al. 1996, Hector et al. 1999), nearly a thousand studies have examined the functional role of biodiversity in terrestrial, freshwater and marine ecosystems (Covich et al. 2004, Cardinale 2011, Cardinale et al. 2012, Gamfeldt et al. 2015). After initially controversial discussions (van der Heijden et al. 1999, Wardle 1999, Hector et al. 2000, Huston et al. 2000), consensus is now emerging that declines in biodiversity have negative consequences for ecosystem functioning (for quantitative data syntheses see Worm et al. 2006, Balvanera et al. 2006, Cardinale et al. 2006, 2011, 2012, Stachowicz et al. 2007, Finkel et al. 2010, Quijas et al. 2010, Hooper et al. 2012, Tilman et al. 2014, Gamfeldt et al. 2015, Hautier et al. 2015). In addition, BEF research has generated new ecological and mathematical theory (Tilman et al. 1997, Loreau and Hector 2001, Pacala and Kinzig 2002, Fox 2005), provided decision support tools for policymakers (Naidoo and Ricketts 2006, Kareiva et al. 2011, InVEST 2016), and led to the establishment of intergovernmental initiatives to preserve global biodiversity (CBD 2010, Larigauderie and Mooney 2010, IPBES 2016).

Despite this progress, early (Hooper et al. 2005) and recent (Cardinale et al. 2012, Tilman et al. 2014, De Laender et al. 2016) BEF consensus papers continue to highlight the need to include the environmental drivers of biodiversity loss into the next generation of BEF experiments. Contrasting BEF relations under stress with the classic BEF protocol of random species loss, and the identification of response and effect traits capable of predicting biodiversity and ecosystem functioning under stress, have been identified among the most urgent need to explore the functional consequences of realistic scenarios of biodiversity change (Suding et al. 2008, Naeem 2008, Cardinale et al. 2012, Tilman et al. 2014, Wardle 2016).

This dissertation, together with other recent efforts to include stressors into BEF theory (see further), has shown that stress can profoundly alter the biodiversity effect on ecosystem functioning. Diversity loss under stress is not **random**, but **selective** according to the species' stress tolerance, and the functional contribution of the most tolerant species plays a key role for functioning under stress. BEF relations in diatom communities under atrazine were steeper than predicted by the classic random design, since the herbicide inhibited the growth of the species (*Nitzschia* sp.) contributing most to energy content and sediment stabilization, and communities were dominated by a tolerant species (*N. arenaria*) contributing little to both processes (Chapter 2). Theory predicts that under stress, the abundance and functional contribution of species can be predicted from their biological traits. The numerical and functional response of diatoms to copper stress was related to the same set of **response and effect traits** (cell size and surface-to-volume

ratio), whilst the diatoms' capacity for mixotrophic growth predicted the numerical but not the functional stress response to atrazine (Chapter 3). **Complementarity**, the main statistical effect used to explain the better performance of species in community, has rarely been quantified under stress and it is unclear what **mechanisms** underpin potential changes in complementarity. Complementarity in diatom communities increased under atrazine and copper stress, driven by **facilitation** (an increased release of extracellular polymers). Complementarity benefited the growth of mixotrophic species (e.g. *C. closterium*) under atrazine, and of copper-sensitive species with low biomass yields (e.g. *A. lineolata*) under metal stress, reducing the net diversity effect on biomass yield in the latter case (Chapter 4). There is little evidence whether stressors drive functioning through **selective effects** (changes in community structure) or **context-dependent effects** (changes in the species' functional contribution), and if both types of stress knock on across **trophic levels**. Selective atrazine effects, leading to dominance by a mixotrophic but lipid-poor species (*C. closterium*), caused a 60% loss of diatom energy content at low atrazine levels. Context-dependent stress effects only affected diatom functioning at high atrazine and copper levels. Both types of stress affected the energy transfer to the next trophic level, causing a concomitant loss in the energy content of their main copepod consumer (*M. littorale,* Chapter 5).

Based on these findings, three main effects that potentially drive functioning in stressed communities can be put forward. This discussion will first focus on **selective stress** effects, i.e. shifts in community structure causing stressed communities to be dominated by species with an above- or below-average functional contribution. Subsequently, context-dependent stress effects on the species' functional contribution will be addressed, which can be mediated by **physiological stress** effects and changes in **complementarity**, i.e. differential interspecific interactions in stressed communities. These three effects are not inherently exclusive, could not always be separated, and will in most cases simultaneously determine the impact of stressor-induced biodiversity changes on ecosystem functioning. Nevertheless, the most important stressor-induced changes in the contribution of diatom communities to ecosystem functioning, including concomitant effects at the consumer level, can be interpreted with regard to these three effects. Each effect is first discussed (sections 6.1 to 6.3) and then examined with regard to its impact on three ecosystem processes (biomass production, sediment stabilization and energy content, section 6.4), before presenting future challenges for BEF research which arise from the outcome of this work (section 6.5).

6.1. Selective stress

Selective stress (Fig. 6.1.1) can be defined as a change in environmental conditions which affects ecosystem functioning by selectively targeting species with a high or low functional contribution (Chapter 1.4.2). Random species loss is the classic protocol in BEF research (Naeem 2008, Cardinale et al. 2012, Wardle 2016). Theory and simulations however predict stress to cause non-random scenarios of biodiversity loss, where species are targeted selectively according to their stress tolerance, and the functional contribution of the most tolerant species plays a key role for the overall functioning in stressed communities (Solan et al. 2004, Bunker et al. 2005, Cardinale et al. 2012).

Here, stressor-induced changes in diatom community structure had little effect on the biomass production of stressed diatom communities, which depended on complementarity (see 6.3) rather than on the biomass production of the dominant species (Chapter 4). Selective atrazine effects reduced the contribution of stressed diatom communities to sediment stabilization, by causing dominance of an atrazine-tolerant species (*N. arenaria*) which however produced little EPS (Chapter 2). When stress-sensitive and stress-tolerant species did not differ in their functional contribution, selective changes in community structure however had no effect on the contribution of stressed diatom communities to sediment stabilization (Chapter 5). The strongest selective stress effects were recorded in terms of diatom energy content. Selective atrazine effects inhibited the growth of a lipid-rich species (*Nitzschia* sp.), causing a disproportionate reduction in the energy content of diatom communities exposed to the herbicide, which was not predicted by the classic design of random species removal (Chapter 2). Similarly, atrazine induced a shift in diatom community structure from dominance by a lipid-rich species (*N. acicularis*) under control conditions towards dominance by a highly mixotrophic, but lipid-poor species (*C. closterium*) under atrazine (Chapter 5). This selective shift in community structure caused a 60% loss in the energy content of diatom communities under atrazine, which furthermore knocked on to the next trophic level and reduced copepod energy content by half, despite invariant diatom biomass and in the absence of any context-dependent stress effects. Selective atrazine stress thus represented the main risk for energy flow at the producer-consumer interface. Conversely, no selective copper effects on diatom energy content were detected, since exposure to the metal did not change diatom community structure (Chapter 5).

Moreover, BEF research has been sparked by concerns about the loss of species and, with few exceptions (e.g. Balvanera et al. 2005, Wittebolle et al. 2009), the majority of BEF experiments have manipulated diversity in terms of species richness, holding evenness constant across richness treatments (Hillebrand et al. 2008, Hillebrand and Matthiessen 2009, Zhang et al. 2012). However, critics often assert that anthropogenic stress is more likely to alter evenness than

richness, with changes in evenness potentially affecting ecosystem functioning before species are driven to extinction (Chapin et al. 2000, Balvanera et al. 2005, Hillebrand et al. 2008). Here, atrazine and copper consistently altered evenness, but did not cause any species loss, highlighting the need to investigate diversity loss not only in terms of species extinctions, but also altered community structure. More importantly, Chapter 2 provided a first experimental evidence that changes in evenness involving the dominance of species with low functional contribution could cause a more important functional loss than changes in species richness.

Selective biodiversity loss can in theory cause more rapid or slower declines in ecosystem functioning compared to random loss scenarios (Ives and Cardinale 2004, Solan et al. 2004, Bunker et al. 2005, Gross and Cardinale 2005), and experimental evidence suggests the functional impact of selective stress depends on the stress tolerance of those species contributing most to functioning (Larsen et al. 2005, McIntyre et al. 2007, Ball et al. 2008, Bracken et al. 2008, Davies et al. 2011, Radchuk et al. 2016). For instance, diversity loss caused by accelerated wave exposure and overfishing reduced nutrient cycling in macroalgae and fish communities respectively to a larger extent than expected from random diversity loss (McIntyre et al. 2007, Bracken et al. 2008). In both cases, wave stress and fishermen targeted the species contributing most to nutrient cycling. In the terrestrial environment, habitat loss caused a disproportionate loss of pollination, because large-bodied insects contributing most to pollination were most sensitive to this type of stress (Larsen et al. 2005). Conversely, insecticide stress had no effect on ecosystem processes in an aquatic food web, since the insecticide only targeted species contributing little to functioning, and the functionally most important species could maintain their functional contribution under stress (Radchuk et al. 2016).

Suding *et al.* (2008) and Hillebrand and Matthiessen (2009) highlighted the need for BEF research to consider the functional consequences of selective biodiversity loss, by proposing a trait-based framework which connects species traits that influence the stress tolerance ('response traits') to species traits that influence ecosystem functioning ('effect traits'). Although experimental tests of this framework are still rare (but see Heuner et al. 2015, Lennon and Lehmkuhl 2016), relating response and effect traits has been identified as a key issue to estimate ecosystem functioning under stress (Cardinale et al. 2012, Wardle 2016).

Here, the capacity of response traits to predict changes in diatom densities and functioning was different depending on stressor type. Changes in diatom cell density ('numerical response') and changes in effect traits ('functional response', in terms of photosynthetic efficiency, energy content and sediment stabilization) were predicted by the same set of morphological traits (cell size and surface to volume ratio). Species which were numerically tolerant to copper performed

best at maintaining functioning when exposed to the metal. A physiological trait (mixotrophy) predicted the diatoms' numerical response to atrazine, but not their functional response. The numerical and functional response to atrazine were not correlated, indicating a limited capacity of tolerant species to maintain functioning under atrazine (Chapter 3). Whilst the response-effect trait framework has been successfully tested in terrestrial habitats, knowledge on traits driving the abundance and functioning of aquatic primary producers along environmental gradients is still scarce. Chapter 3 allowed to identify morphology and mixotrophy as key drivers if microalgal performance along gradients of metal and pesticide stress, corroborating findings by Larras et al. (2014) who also found mixotrophy to drive diatom cell densities in the presence of herbicides. By relating changes in density to changes in effect traits, chapter 3 provides a novel exntension to existing trait-based approaches, since changes in effect traits have not been incorporated yet within response-effect trait frameworks.

The diatoms' numerical stress response to atrazine and copper corresponded to the community structure observed under both stressors: copper-exposed diatom communities were dominated by *Entomoneis* sp. and *N. acicularis* (Chapters 4 & 5) which were among the numerically and functionally most tolerant species to the metal. Consequently, the functioning of diatom communities was only affected at high (500 µg/L) copper concentrations. Atrazine-exposed diatom communities were dominated by two species which were numerically tolerant to the herbicide (*N. arenaria* and *C. closterium*). These species contributed seven times less to sediment stabilization and respectively fifteen and fourty times less to energy content than the atrazine-sensitive species which had dominated communities under unstressed conditions (*Nitzschia sp.* and *N. acicularis*, Chapter 2 and 5), causing the negative selective atrazine effects on diatom functioning (see above). *Entomoneis* sp. and *C. closterium* were the most copper- and atrazine-tolerant species among a set of 17 diatom species, and were characterized by the largest cell size and lowest surface-to-volume-ratio, and by the highest capacity for mixotrophic growth respectively (Chapter 3).

Whilst the above examples confirm the potential of traits to predict changes in community structure and their effect on functioning under stress (Hillebrand and Matthiessen 2009), this trait-based approach could not consistently predict community structure under stress: For instance, *N. arenaria* reached high relative abundances in atrazine-exposed communities (Chapter 2), despite having no capacity for mixotrophic growth (Chapter 3). Similarly, the good performance of *N. acicularis* in copper-exposed communities (Chapter 5) was not predictable from its traits, since this species was neither characterized by a large cell size nor by a low surface to volume ratio (Chapter 3). Selective stress effects on diatom community structure and functioning were thus consistently predicted by the species' tolerance (EC50 values), but not

always by the species' traits, arguably because this work did not include other traits relevant to microalgal stress tolerance. Such traits involve metal-binding metallothioneins and phytochelatins, or the structure of the quinone binding site in photosystem II, which determine algal tolerance to copper and atrazine respectively (Galloway and Mets 1984, Gekeler et al. 1988, Robinson 1989, Fernandes and Henriques 1991, Galgani et al. 1999). Furthermore, stress tolerance in monoculture, which was predicted by response traits, could be decoupled from stress tolerance in community when species benefited differentially from complementarity. For example, in the diatom communities in Chapter 4, *A. lineolata* was the least copper-tolerant species in monoculture, which could be expected based on its response traits (smallest cell size, largest surface to volume ratio of all component species). *A. lineolata* however benefited most from an increase in complementarity and thus performed well in communities exposed to the metal (but see 6.3), thus limiting the capacity of response traits to predict community structure under stress.

Nevertheless, the identification of key morphological and physiological traits determining, in part, the structure and functioning of primary producer communities under stress, highlighted the value of trait-based approaches to predict the consequences of selective stress for ecosystem functioning. In ecotoxicology, approaches relating traits to stress tolerance have been tested (Baird and Van den Brink 2007, Rubach et al. 2012, Larras et al. 2014) and trait-based bioindicator tools are being used to link the pesticide load in European waters to the structure of aquatic communities (Liess and Von Der Ohe 2005, Beketov et al. 2009). In BEF research, the theoretical framework for the incorporation of trait-based approaches has been set (Suding et al. 2008, Hillebrand and Matthiessen 2009, Litchman et al. 2015). Methodological advances now allow for time-efficient quantifications of traits at the individual level (Fontana et al. 2014), and recent experimental approaches have used response and effect traits to understand the structure and functioning of communities under stress (Koide et al. 2014, Heuner et al. 2015, Zwart et al. 2015, Lennon and Lehmkuhl 2016). These theoretical and methodological advances should allow to progressively complement the classic design of random species loss with trait-based approaches that more realistically reflect community structure and ecosystem functioning under anthropogenic change.

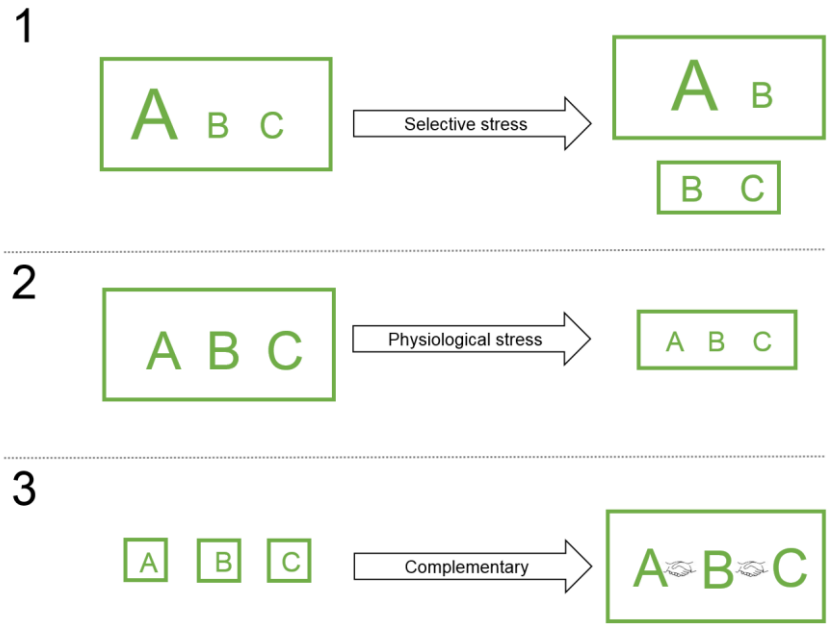


Fig. 6.1: Scheme indicating three effects which potentially drive functioning in stressed communities. For the sake of simplicity, this scheme uses communities with only three species (A, B and C). The font size of A, B and C indicates the species' functional contribution. Communities are marked by green boxes, with the size of the box marking the community's functional contribution. **Panel 1** shows **selective stress** (for details see Chapter 1.4.2), which occurs when the species contributing most to functioning are also least stress-tolerant. The example community in Panel 1 includes a species with a high functional contribution (species A) and two species with a low functional contribution (species B and C). Selective stress has limited effect on community functioning, if the stressor does not remove species A, which contributes most to functioning (upper community on the right-hand side of panel 1). Selective stress disproportionately reduces community functioning, if the stressor removes species A (lower community on the right-hand side of panel 1). **Panel 2** shows **physiological stress** (for details see Chapter 1.4.3), which affects ecosystem functioning when stress alters the species' functional contribution. For the sake of simplicity, Panel 2 indicates a community in which the species, A, B and C contribute equally to functioning, with each species' functional contribution altered to the same extent by stress. **Panel 3** shows **complementarity** (for details see Chapter 1.6), which occurs when species contribute more to functioning when grown in community than in monoculture. Facilitation and niche partitioning are the main mechanisms driving complementarity. In contrast to selective and physiological stress, complementarity is not a 'stress effect', and can operate under stressed and unstressed conditions. The example in Panel 3 shows three species A, B and C with a low functional contribution when being exposed to stress in monoculture, and an increased functional contribution when being exposed to stress in community due to facilitative interactions.

6.2. Physiological stress

Physiological stress can be defined as stress which affects ecosystem functioning by directly altering the species' functional contribution (Fig. 6.1.2). Direct stress exposure to anthropogenic stressors is rarely included into the design of BEF experiments (McMahon et al. 2012, Steudel et al. 2012), which remove species to mimic stressed ecosystems, but assume that the functional contribution of the species in these low-diversity-communities remains unchanged. However, stressors that cause species loss can impose a physiological cost on the surviving species, e.g. acclimation by allocating resources from growth to survival pathways, which can affect the contribution of stressed communities to ecosystem functioning (Schimel et al. 2007, Hawlena and

Schmitz 2010). This has resulted in calls to consider sublethal physiological effects on the species' functional contribution, when estimating ecosystem functioning under stress (Relyea and Hoverman 2006).

In all chapters, atrazine and copper reduced diatom growth and thus biomass production. The main physiological mechanisms through which atrazine and copper affect diatom growth comprise an inhibition of electron transport in photosystem II (atrazine, Dorigo and Leboulanger 2001) and the damage of cell membranes, thylakoids and chloropolasts (copper, Knauert and Knauer 2008, for more details see Chapter 1.8.1). Conversely, at low atrazine and copper levels the diatoms' contribution to sediment stabilization could be stimulated through an increased EPS production (Chapters 3-5), whilst high stress levels resulted in an EPS production at or below control levels (Chapters 2-3 and 4-5 respectively). Next to its important functional role in sediment stabilization (De Brouwer et al. 2000, Decho 2000), the excretion of EPS also represents a physiological stress response of benthic diatoms to metal, nutrient and salinity stress, which can explain the stimulated EPS secretion at low stress levels (Pistocchi et al. 1997, Liu and Buskey 2000, Staats et al. 2000a, Levy et al. 2007). The release of EPS from diatom biofilms is moreover age-dependent, with diatoms maximizing their *per capita* EPS production during early stages of the growth phase (Myklestad et al. 1989, De Troch et al. 2012b). However, EPS production cannot be maintained when photosynthesis and thus carbon supply for EPS biosynthesis is inhibited (Staats et al. 2000b, Mason et al. 2003). The age of diatom cells (EPS measurement in exponential rather than stationary growth) and the inclusion atrazine- and copper-tolerant species (e.g. *Entomoneis* sp., *N. acicularis*, *Gyrosigma* sp.) might explain the capacity of diatom communities to maintain their EPS at control levels in the high stress treatments of Chapters 4 and 5. An inhibition of the photosynthetic carbon supply for EPS biosynthesis on the other hand can explain why diatom cells could not increase (Chapters 4-5) or maintain (Chapter 2-3) their EPS production with increasing stress levels. Diatom energy content was consistently reduced by both stressors, with diatom cells producing less fatty acids at stress levels where their growth was still unaffected (Chapters 2-5). Several physiological pesticide and metal effects can lead to such losses in algal energy content: a reduced carbon supply for fatty acid synthesis (when photosynthesis is inhibited, Guschina and Harwood 2006), the conversion of unsaturated to saturated fatty acids (Chia et al. 2013a, 2013b), metal-induced lipid peroxidation (Stohs and Bagchi 1995, Chaoui et al. 1997, Mehta and Gaur 1999, Li et al. 2006) or the inhibition of enzymes involved in fatty acid synthesis (Böger et al. 2000).

To what extent did these physiological effects impact the contribution of stressed diatom communities to ecosystem functioning? Linking physiological responses to environmental stress to processes at the ecosystem level has been a major theme in plant ecology (Grime 1977, Chapin

et al. 2002). The few BEF experiments conducted under stressful conditions could not disentangle whether changes in ecosystem functioning were mediated by biodiversity loss, or by effects of environmental disturbance on the species' functional contribution (Steudel et al. 2012, Radchuk et al. 2016, but see Fox and Harpole 2008, Baert et al. 2016).

Stressors can affect ecosystem functioning through selective biodiversity loss (e.g. by targeting species with a high functional contribution, see 6.1), or through 'context-dependent effects', i.e. changes in the species' functional contribution (Fox 2006, Fox and Harpole 2008, Tylianakis et al. 2008, Hiddink et al. 2009). These changes in functional contribution can arise from direct effects of environmental drivers (Fox and Harpole 2008), i.e. the here-described physiological effects, as well as from species interactions (e.g. a change in complementarity, Fox 2006, Fox and Harpole 2008)

Here, selective and context-dependent stress effects could be disentangled by contrasting functioning (energy content and sediment stabilization) in stressed diatom communities with that expected from unstressed monocultures (Chapters 2 & 5). The increased contribution to sediment stabilization (EPS production) of diatom communities under copper and atrazine was driven by positive context-dependent effects (Chapter 5). Since physiological effects of both stressors also increased the EPS production of most diatom species in monoculture (see above), it can be assumed that the increased EPS production of stressed diatom communities represented a direct physiological stress response. Nevertheless, the extent to which species interactions contributed to this increased EPS production under stress could not be quantified. No selective stress effects on EPS production were recorded when species did not differ in their functional contribution (Chapter 5). When species however differed in their functional contribution and selective stress caused dominance by an unproductive species (*N. arenaria*, Chapter 2), the resulting decline in EPS production could not be compensated by any context-dependent effects.

Context-dependent stress effects generally reduced diatom energy content, with the fatty acid production of most species being reduced at stress levels which did not affect other processes. In Chapter 5, this loss of diatom energy content also knocked on to the consumer level and caused a concomitant loss in copepod energy content. However, compared to selective stress effects, context-dependent effects were of minor importance for diatom energy content. By promoting dominance of tolerant but lipid-poor species, selective atrazine effects caused a more important loss of diatom energy content than the context dependent effects of both stressors, at chemical concentrations where no context dependent effects on diatom energy content were recorded. At atrazine levels which, on average, reduced diatom fatty acid content by half (150 µg/L, see EC50 values in Chapter 3), the herbicide caused a shift in diatom community structure involving

dominance by tolerant species (*C. closterium* and *N. arenaria*) producing fifteen and fourty times less fatty acids than those dominating under unstressed conditions (*Nitzschia* sp., *N. acicularis*).

When species showed large differences in their functional contribution, selective stress effects via changes in community structure were thus a more potent threat to ecosystem functioning than stress effects on the species' functional contribution. Physiological and selective stress effects have rarely been compared in BEF research, mainly because most BEF experiments do not include direct stress exposure and thus exclude physiological stress effects (McMahon et al. 2012, De Laender et al. 2016). Conversely, community ecotoxicology has focused on contrasting physiological and selective stress effects on diversity and functioning, with the impact of selective shifts in community structure often exceeding that of direct adverse stress effects (Rohr and Crumrine 2005, Relyea and Hoverman 2006, Rohr et al. 2006, Clements and Rohr 2009). Previous work also found that whilst physiological stress effects commonly affect ecosystem processes on the short term, selective changes in community structure can have strong long-term effects on functioning, which prevail after the stressful conditions (Woin 1998, Schimel et al. 2007, Suding et al. 2008). Moreover, physiological stress effects on the functional contribution of diatoms in monoculture could, in part, be compensated by positive species interactions in stressed communities, as will be discussed in the next section.

6.3. Complementarity

Complementarity effects occur when species contribute more to functioning in community than in monoculture (Fig. 6.1.3), and result from ecological mechanisms such as niche partitioning or facilitative interactions between species (Loreau and Hector 2001, Fox 2005). Experimental evidence linking these mechanisms to complementarity effects is however still scarce, and the extent to which these mechanisms broadly contribute to ecosystem functioning has yet to be confirmed (Vanelslander et al. 2009, Carroll et al. 2011, Cardinale et al. 2011, 2012). Trait-independent complementarity occurs when niche partitioning or facilitative interactions benefit all species, whilst trait-dependent complementarity occurs if these mechanisms are not vice-versa and favour species with a high or low functional contribution (Fox 2005, Hector et al. 2009).

As such, complementarity is not a 'stress effect', but operates under unstressed conditions, and has been in the focus of major debates in BEF research (Huston 1997, Huston et al. 2000, Cardinale et al. 2006, Fargione et al. 2007, Hodapp et al. 2016). Most of these debates have centred on whether biodiversity effects on ecosystem functioning are driven by complementarity effects, or by the competitive dominance of single, highly productive species (dominance effects, but see Chapter 1.6 and Loreau and Hector 2001, Fox 2005). Over the last decade, BEF research has shown that complementarity and dominance effects are not inherently exclusive and jointly control

ecosystem functioning (Hector et al. 2009, Cardinale et al. 2012, Hodapp et al. 2016). On average over many ecosystems, each effect contributes roughly 50% of the biodiversity effect on functioning, whilst complementarity appears to be the main effect driving BEF relations in aquatic ecosystems (Cardinale et al. 2011, 2012).

There is still little evidence on how complementarity is affected by anthropogenic stress (but see Fernandes et al. 2011, Wang et al. 2013, Baert et al. 2016). Potential changes of complementarity are however crucial for determining how stress will alter species' contribution to ecosystem functioning. If complementarity is not affected by stress, i.e. species interactions are the same under stressed and unstressed conditions, stress should affect the BEF relation only through selective biodiversity loss and physiological effects. The biodiversity effect on functioning would thus depend on the functional contribution of the most stress-tolerant (and thus dominant) species (Baert et al. 2016). If, however complementarity becomes more positive under stress, e.g. through an increase in facilitative interactions, adverse selective and physiological stress effects on functioning could be compensated. This potential for functional compensation among stressed communities has thus been a major subject of discussion BEF research (Fox 2006, Duffy et al. 2007, Fox and Harpole 2008) and stress ecology (Bertness and Callaway 1994, Maestre et al. 2009, 2010, He et al. 2013).

Here, diatoms produced more biomass when grown in community than in monoculture. This positive biodiversity effect was caused by complementarity, which increased under stress (Chapter 4). Atrazine and copper reduced diatom biomass in monoculture (see 6.2), but this loss of biomass could partially be compensated by an increase in complementarity when diatoms were exposed to the same stress levels in community. Facilitative mechanisms can drive complementarity effects in unstressed conditions, for example in legumes and insects (Cardinale et al. 2002, Temperton et al. 2007). In microalgae, the release of organic exudates can act as a facilitative mechanism driving complementarity effects (Vanelslander et al. 2009). At present, there is however no ecophysiological evidence linking changes in complementarity under stress to changing facilitative mechanisms. Under atrazine and copper stress, diatom communities increased their EPS production which, in part, predicted increases in complementarity under stress, providing a first ecophysiological evidence for a facilitative mechanism as driver of complementarity under stress (Chapter 4). This role of EPS as a 'sleeping' facilitative mechanism, which when stimulated under stress predicted changes in complementarity highlights the two roles of microalgal EPS production: first, as an ecosystem process *per se*, promoting sediment stabilization (De Brouwer et al. 2000, Decho 2000, Gerbersdorf et al. 2009a). Second, as a physiological stress response which not only operates in monoculture (Pistocchi et al. 1997, Staats

et al. 2000a), but also acts as driver of complementarity effects which helps to maintain biomass production under stress.

Moreover, complementarity under stress was largely trait-dependent, and benefited species depending on their properties: under atrazine and copper respectively, complementarity favoured the growth of mixotrophic (*C. closterium*, *N. acicularis*, *Nitzschia* sp.) and copper-sensitive species (*A. lineolata*, *N. digitoradiata*, *Gyrosigma* sp.). To date, BEF experiments which partitioned trait-independent and trait-dependent complementarity found only minor contributions of trait-dependent complementarity to the net biodiversity effect on ecosystem functioning (Hector et al. 2009, Fernandes et al. 2011, Long et al. 2013, Stachova et al. 2013, Siebenkäs et al. 2016). Here, copper-sensitive species were characterised by low biomass yields, causing a negative trait-dependent complementarity under copper stress, which largely offset positive trait-independent complementarity effects and thus limited the biodiversity effect on diatom biomass production. The release of extracellular polymers as a facilitative mechanism thus predicted changes in complementarity in stressed diatom communities, but was not necessarily beneficial for ecosystem functioning under stress when complementarity benefited species with a low functional contribution. In total however, complementarity was consistently positive, increased under stress when trait-dependent complementarity did not favour species with a low functional contribution, and thus represented the key driver of diatom biomass production under stress.

6.4. Multifunctionality

The large majority of BEF research has focused on one ecosystem process at a time, most commonly the biomass production of primary producers (Balvanera et al. 2006, Jiang et al. 2008, Cardinale et al. 2011). This focus on biomass is understandable, given the importance of biomass production for e.g. food and material production or carbon sequestration, which represent important ecosystem services that human society heavily relies on (Jiang et al. 2008, Cardinale et al. 2012). Here, however, different species contributed to different ecosystem processes (see 6.1-6.3). These results correspond to a growing body of BEF studies which found that sustaining multiple rather than single ecosystem processes requires a greater number of species, and suggested that more biodiversity is needed to maintain the 'multifunctionality' of ecosystems (Isbell et al. 2011, Maestre et al. 2012, Pasari et al. 2013, Lefcheck et al. 2015, Soliveres et al. 2016).

If the covariation among diversity effects driving different ecosystem processes is strong, e.g. if multiple processes are regulated by either complementarity or the presence of one dominant species, a subset of diversity should be sufficient to sustain multifunctionality (Cardinale et al. 2011). This principle also applies to the maintenance of multifunctionality under stress. If the

diversity-multifunctionality relation is regulated by similar stress effects, e.g. by an increase in complementarity or by selective stress effects on the dominant species, focussing on one of these effects allows to predict multiple ecosystem processes under stress. If different ecosystem processes are driven by different stress effects, the maintenance of a particular ecosystem process under stress might reduce other processes, and multifunctionality imposes a tradeoff for the conservation of ecosystem functioning under stress. Here, for example, a facilitation-driven increase in complementarity stimulated the growth of a highly mixotrophic but lipid-poor species (*C. closterium*). This allowed to maintain diatom biomass under herbicide stress, but came at the cost of a negative selective stress effect on diatom energy content.

The functional impact of selective biodiversity loss relative to changes in complementarity increases with differences in the species' functional contribution (Jiang 2007): when species contribute similarly to functioning, selective biodiversity loss can be compensated by an increase in complementarity effects, whilst this potential for compensation decreases when biodiversity loss selectively targets highly productive species. Complementarity commonly drives biomass production, but tends to have weaker effects on certain biochemical or trophic processes (Duffy 2002, Jiang et al. 2008, Cardinale et al. 2011). Processes such as decomposition, trophic energy transfer, nutrient cycling, bioturbation or ocean carbon export can instead depend on the presence of organisms with a disproportionately high contribution to functioning (Emmerson et al. 2001, Hillebrand and Cardinale 2004, Waldbusser et al. 2004, Solan et al. 2004, Jiang 2007, Jiang et al. 2008, Cardinale et al. 2011, Bracken and Low 2012, Van Colen et al. 2012, Litchman et al. 2015).

Here, differences in functional contribution amongst diatom species were limited in terms of biomass production, and most pronounced in terms of energy content. The monoculture biomass of the most productive species (*Nitzschia* sp. in Chapter 2, *N. arenaria* in Chapters 4 and 5, respectively) exceeded that of the least productive species (*S. robusta* and *A. lineolata*) by a factor three and four, respectively. The species contributing most to sediment stabilization (*Nitzschia* sp. and *A. lineolata* in Chapters 2 and 5, respectively) produced seven times more EPS than the least productive species (*S. robusta* and *N. arenaria*). The most lipid-rich species (two different *Nitzschia* sp.) however produced fourty and fifteen times more fatty acids than the most lipid-poor species in the diatom communities of Chapters 2 and 5 (*N. arenaria* and *C. closterium*).

In accordance with these differences in functional contribution, biomass production did not depend on the identity of the dominant species, and was reduced by physiological atrazine and copper effects, which could however be compensated by positive complementarity effects on stressed diatom communities (Fig. 6.2 A). The extent to which complementarity could compensate

stress effects on diatom biomass production depended on the stress level, and whether complementarity favoured the growth of low- or high-yield species. For energy content and sediment stabilization, complementarity effects could not be quantified, since additive partitioning would require information on the fatty acid and EPS production of every species in monoculture and in community. Fig. 6.2 consequently does not make a statement on complementarity as driver of diatom energy content and sediment stabilization under stress.

EPS was affected by selective stress, when stress-sensitive and tolerant species differed in their functional contribution. At low stress levels, diatoms increased their EPS production as a physiological stress response, whilst at high stress diatoms could not maintain their EPS production (Fig. 6.2 B). In a best-case scenario, stress thus did not target the functionally most important species, diatoms increased their EPS production at low stress, and stressed diatom communities produced more EPS than under control conditions. In a worst-case scenario, stress targeted the most productive species and diatoms could not maintain their EPS production under high stress, causing a disproportionate loss of EPS production in stressed diatom communities (Fig. 6.2 B).

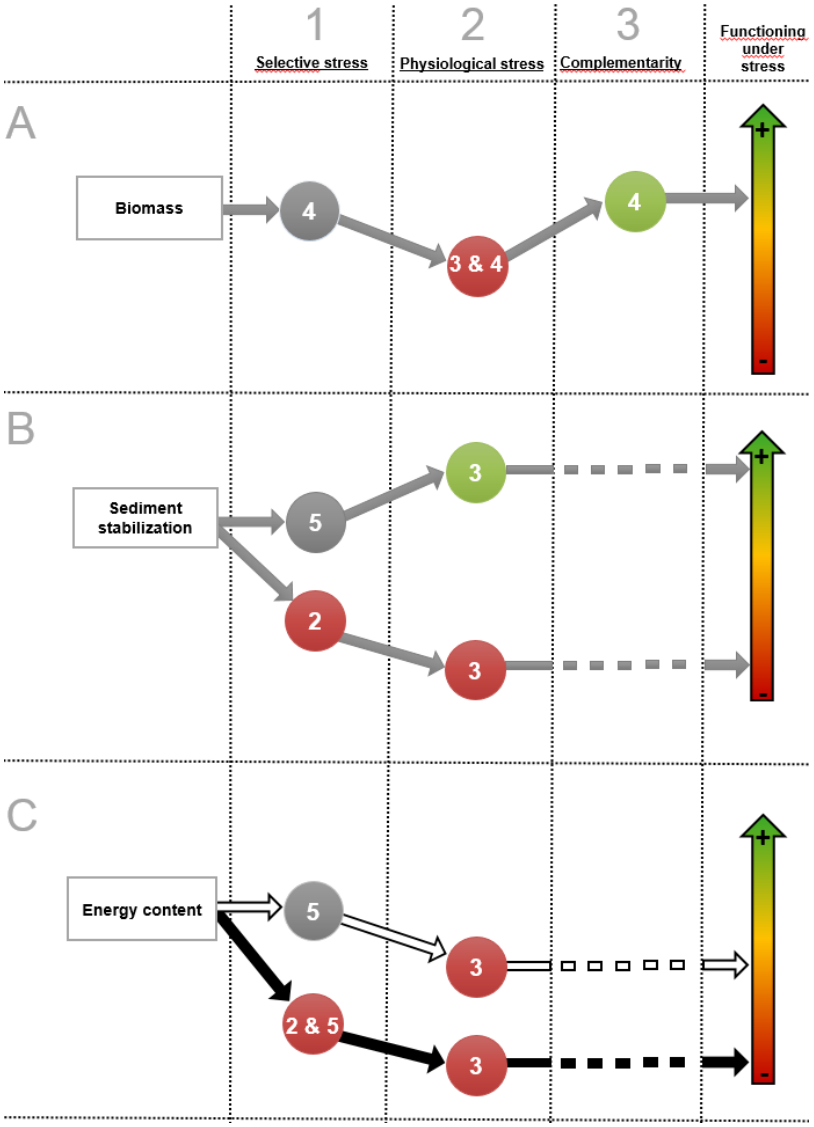


Fig. 6.2: Summary of three effects (Selective stress – Panel 1, Physiological stress – Panel 2, Complementarity – Panel 3) determining the contribution of diatom communities to three ecosystem processes under stress (Biomass production – Panel A, Sediment stabilization – Panel B, Energy content – Panel C). Red, grey and green circles respectively represent negative, neutral and positive impacts of each effect on ecosystem processes. The numbers within the circles indicate the chapters in which the respective effects were analysed (Chapters 2 to 5). Black, white and grey arrows denote effects by atrazine, copper and both stressors respectively. The coloured arrows on the right-hand side of panels A, B and C represent an approximation to which extent stressed diatom communities could maintain their contribution to ecosystem functioning (green: high functioning under stress; red: low functioning under stress). For the sake of simplicity this figure does not present the extent of ecosystem functioning change in the different treatments of Chapters 2 to 5. Instead, this figure has to be understood as a qualitative rather than quantitative interpretation of the impact of selective stress, physiological stress and complementarity on the contribution of stressed diatom communities to ecosystem functioning. Whilst the impact of selective stress, physiological stress and complementarity on diatom biomass production were consistently neutral, negative and positive respectively, changes in diatom energy content and sediment stabilization differed depending on stress type (atrazine or copper) and stress level. For diatom energy content and sediment stabilization, this figure thus indicates a best-case and worst-case scenario, resulting from the combination of selective and physiological atrazine and copper effects on the functional contribution of diatom communities.

Diatom energy content was most affected by selective atrazine stress, due to the large differences in the species' fatty acid production (but see above). As predicted from the species' EC50 values, the most copper-tolerant species also produced most fatty acids, and selective copper stress had no effect on diatom energy content. Compared to biomass production and sediment stabilization, diatom energy content was also most affected by physiological stress, with the fatty acid production of most species being reduced at stress levels which did not affect other processes. Context-dependent effects of both stressors thus also caused a loss of diatom energy content, even in the absence of selective stress effects (Fig. 6.2 C). In a best-case scenario, diatom energy content was not affected by selective copper stress, and only reduced by context-dependent effects at high copper levels. In a worst-case scenario, low atrazine levels reduced diatom energy content through selective changes in community structure, whilst high levels of the herbicide further reduced diatom energy content through context-dependent effects.

These strong effects of chemical stress on diatom fatty acid content which, partly despite invariant biomass, knocked on to the next trophic level highlight the relevance of diet quality in a trophic BEF context. Trophic BEF research has mostly focussed on diet quantity, i.e. the biomass production of primary producers (Balvanera et al. 2006, Duffy et al. 2007), whilst stress-induced changes in diet quality have not been analysed yet in a BEF context. Diet quality received broad attention in marine trophic ecology when population declines of some well-known marine top predators could be explained by changes in the quality of their diet. For example, reproductive failures in seabirds (Wanless et al. 2005, Kitaysky et al. 2006, Österblom et al. 2008) and declines in sea lion populations (Anderson and Piatt 1999, Rosen and Trites 2005, Trites et al. 2007) have been linked to losses in the energy content of their diet, and to shifts in prey composition from lipid-rich to lipid-poor prey species.

However, diet quality does not only play a crucial role at the top of the food web, but is the key driver of energy transfer at the primary producer-consumer interface. In aquatic ecosystems, algal diet quality is highly sensitive to changing environmental conditions, and energy content shows considerably variation among and within algal classes (Dunstan et al. 1993, Taipale et al. 2013, Guo et al. 2016). Community shifts from high- to low-energy primary consumers can cause trophic decoupling, i.e. low energy transfer efficiencies to the consumer level despite high producer biomass, which reduces consumer energy content and ultimately reproduction (Müller-Navarra et al. 2000, Guo et al. 2016). The limited duration of the experiments in this work allowed to test for the former (energy transfer) but not the latter (consumer reproduction) consequence of altered diet quality. Nevertheless, anthropogenic change is increasingly altering the energy content of primary producers (Teoh et al. 2013, Guo et al. 2016) and is causing large-scale shifts in both local and global phytoplankton compositions (e.g. from energy-rich diatoms to energy-

poor cyanobacteria, Bopp et al. 2005, Moran et al. 2010, Litchman et al. 2015). Evaluating the functional impact of these changes not only in terms of diet quantity, but also diet quality and trophic energy transfer will be major task for future BEF research.

The multiple ecosystem processes measured in this work should however not be directly equated to ecosystem functioning. Whilst biovolume has become a largely consistent measure of microalgal biomass (Hillebrand et al. 1999), the contribution of diatoms to diet quality, sediment stabilization and primary production is related to respectively fatty acid content, EPS production and photosynthetic efficiency, but also to other processes as will be discussed further. Essential fatty acids are a main determinant for primary producer diet quality. The quality of diatoms as food for primary consumers however also depends on the presence of mechanical (Hamm et al. 2003, Pondaven et al. 2007) and chemical protection mechanisms (Cembella 2003, Pohnert 2004, 2005, Leflaive and Ten-Hage 2007), for instance polyunsaturated aldehydes and other fatty acid degradation products which inhibit copepod reproduction (Pohnert et al. 2002, Leflaive and Ten-Hage 2007, Ianora and Miralto 2010). Algal nutrient stoichiometry (Brett et al. 2000, Cross et al. 2003, Lau et al. 2005) as well as consumer ingestion and digestion rates (Cowie 1996, Paffenhöfer and Köster 2005, De Troch et al. 2006, 2007) further mediate trophic energy transfer.

Similarly, microalgal EPS production is a strong driver of sediment stability in muddy intertidal environments (Decho 1990, 2000, Underwood and Paterson 2003), but diatom EPS is a heterogeneous mixture of carbohydrates, which represent the main bulk of diatom EPS production, but also proteins and nucleic acids (Flemming and Wingender 2001, Stal 2003). Diatoms produce two operationally defined fractions of EPS, bound and soluble EPS, which are respectively stored inside or in close association to diatoms cells, or excreted into the surrounding medium (De Brouwer et al. 2002, Stal 2003). Bound EPS are rich in neutral sugars, mainly glucose, are used as intracellular carbohydrate storage and are often metabolized by the diatoms themselves (De Brouwer et al. 2002, Stal 2003). Soluble EPS are characterised by a lower glucose content, are rich in uronic acids and other acid sugars, and due to their highly charged nature are characterised by a strong binding affinity to silt and clay particles (Stal 2003). Soluble EPS have therefore been reported as the main driver of biogenic sediment stabilisation (De Brouwer 2002, Underwood and Paterson 2003), and were therefore chosen as measurement endpoint in this work. However, the contribution of diatom species to biogenic sediment stabilization is driven not only by the absolute amount of soluble EPS produced, but also by the relative amount of uronic acids within the soluble EPS fraction, which should be the focus of future studies comparing the contribution of different diatom species to biogenic sediment stabilization (De Brouwer et al. 2005). More generally, EPS secreted by diatoms are a labile organic carbon source that may be degraded or modified by bacteria (van Duyl et al. 1999, De Brouwer et al. 2005) and since diatom

EPS are watersoluble they probably partially removed with every tide (Stal 2003). Moreover, bacteria also contribute to the EPS pool in muddy intertidal habitats (Dade et al. 1990, Lubarsky et al. 2010) and sediment stability depends not only on EPS but also other properties such as sediment grain size (Tolhurst et al. 1999, Stal 2003, De Brouwer et al. 2005) and bioturbation (de Deckere et al. 2001, Fernandes et al. 2006).

Microalgal primary production is increasingly estimated from chlorophyll fluorescence measurements (Kromkamp et al. 1998, Consalvey et al. 2005). Photosynthetic efficiency as estimated by pulse-amplitude fluorescence measurements correlates with microalgal carbon fixation and oxygen production, this correlation can however show considerable variation (Kroon 1994, Hartig et al. 1998b, Barranguet and Kromkamp 2000). Notably, the relation between photosynthetic efficiency and primary production loses its linearity at high irradiances (Kromkamp and Peene 1999, Barranguet and Kromkamp 2000) and due to alternative electron sinks which may consume oxygen, such as the Mehler reaction, photorespiration or the photsynthetic reduction of nitrate by nitrate reductase (Kromkamp et al. 1998, Flameling and Kromkamp 1998, Barranguet and Kromkamp 2000). Photosynthetic efficiency based on electron transport in photosystem II as determined by pulse-amplitude fluorometry can thus serve as an estimate for primary production, to determine absolute production rates this method should however be combined with other techniques, such as measuring oxygen evolution with microelectrodes or measuring the carbon entering photosynthesis with ^{14}C radiotracers (Underwood and Kromkamp 1999, Consalvey et al. 2005).

The stress-induced changes in ecosystem processes should be interpreted within the context of this work's experimental design. Specifically, the usage of xenic diatom cultures implies that bacterial growth was not controlled for in the experimental microcosms. Bacteria commonly grow in tight association to marine microalgae and can influence the growth of diatoms through both synergistic and antagonistic interactions (Grossart 1999, Kaczmarska et al. 2005, Jung et al. 2008, Gärdes et al. 2011, Amin et al. 2012). Bacteria promote diatom growth through the remineralization of organic matter (Cho and Azam 1988, Bruckner *et al.* 2008; Fouilland 2012; Novoveská *et al.* 2016), including EPS produced by diatoms (Hayes et al. 2007), with bacterial exoenzymes breaking down complex EPS molecules which are not transportable across cell membranes, thereby stimulating the growth of mixotrophic diatom species (Tuchman et al. 2006). Bacteria moreover stimulate diatom growth through the production of vitamins (Hayes and Guillard 1974, Croft et al. 2005), the post-mortem recycling of silica in diatom frustules (Azam and Worden 2004), the excretion of iron-binding siderophores (Amin et al. 2012), or by detoxifying byproducts generated during diatom metabolism (Hünken et al. 2008). Antagonistic interactions between bacteria and diatoms include the release of algicidal compounds by bacteria (Mayali and

Azam 2004), which diatoms can counter through the release of antibacterial compounds (Ribalet et al. 2008), as well as competition for nutrients (Thingstad et al. 1993, Guerrini et al. 1998, Risgaard-Petersen et al. 2004). Chemical stressors are causing shifts not only in microalgal, but also in bacterial community structures (Rois-Marshall et al. 2013, Ponsati et al. 2016), which could potentially affect diatom-bacteria interactions and the contribution of diatoms to ecosystem processes. The refreshing of growth medium during the experiments may have limited the importance of bacteria as recyclers of nutrients for diatom growth, as well as diatom-bacteria competition for limiting nutrients. In field conditions, the strength of interspecific interactions will however increase with resource limitations and environmental fluctuations (Stachowicz et al. 2008a, making interactions with bacteria a driving force of microalgal contribution to ecosystem functioning (Naeem et al. 2000, Morin and McGrady-Steed 2004).

Moreover, the setting of the experiments in controlled laboratory microcosms allowed to identify the key mechanisms driving the BEF relation, but makes it hard to generalize the outcome of these experiments to field conditions. In natural systems, diatom biofilms have to cope with a broad range of abiotic conditions such as salinity (Underwood et al. 1998, Sahan et al. 2007) and nutrient gradients (Underwood et al 1998, Hillebrand et al. 2000), desiccation (Souffreau et al. 2010, McKew et al. 201), grazing pressure (Hillebrand et al. 2000, Thompson et al. 2004, Sahan et al. 2007), and rapidly changing and often extreme temperature and light conditions (Souffreau et al. 2010, Salleh and McMinn 2011, Teoh et al. 2013, Barnett et al. 2015). In natural systems, such fluctuating abiotic conditions could represent a tradeoff to the capacity of diatom communities to cope with metal and pesticide stress. Diatoms are the main primary producers in the muddy North Sea intertidal (Forster et al. 2006), but tend to show a higher tolerance to atrazine and copper pollution than other classes of microalgae (Brand et al. 1986, Guasch et al. 1998, DeLorenzo et al. 2001, Leboulanger et al. 2001, Miao et al. 2005). The metal and herbicide stress on algal diversity and ecosystem functioning could thus be more than the reported in this work in systems in which microalgae other than diatoms represent the main primary producers. Moreover, harpacticoid copepods show a higher grazing efficiency in the absence of sediment, as the sediment could hinder the functioning of their feeding apparatus (De Troch et al. 2006). Under field conditions, copepods could thus have a more limited ability to compensate for stressor-induced losses in diet quality through compensatory feeding. Complementarity effects, which emerged as a main driver of diatom biomass production under stress, also tend to increase with environmental heterogeneity and environmental fluctuations (Špaèková and Lepš 2001, Cardinale et al. 2007, Stachowicz et al. 2008b, Hodapp et al. 2016).

Whilst the experimental setup could thus have underestimated diversity-functioning relations compared to natural systems, it should be highlighted that in their natural habitat, diatom biofilms

are unlikely to be exposed to the levels of metal and pesticide stress used in this work. In polluted estuaries in North America, Asia, the middle east and Oceania, reported atrazine and copper levels are still representative or even exceeding the concentrations used in this work (Pennington et al. 2001, Bejarano et al. 2005, Alyahyaet al. 2011, Bai et al. 2011, Smith et al. 2012). In Europe, copper and atrazine are still frequently detected in coastal ecosystems, although atrazine has been banned in 2004 (Noppe et al. 2007, Van Sprang et al. 2007, Loos et al. 2009, Janssen et al. 2010, Nödler et al. 2013). The reported field concentrations of both chemicals nevertheless represent stress levels which do not lead to microalgae and copepod mortality, making chemical-stress-induced losses of diatom or copepod species in North Sea intertidal systems highly unlikely (Plumley and Davies 1980, Brand et al. 1986, Peterson et al. 1994, Hall et al. 1995, DeLorenzo et al. 2001, Bejarano and Chandler 2003, Miao et al. 2005, Pérez et al. 2006, Debelius et al. 2008, Knauert 2008, Masmoudi et al. 2013, Wood et al. 2014). However, one of the main outcomes of this work is that if losses in ecosystem functioning were observed, these were mainly due to shifts in community structure towards dominance by unproductive species, rather than species loss or direct stress effects on the species' functional contribution (but see above). Such shifts in community structure can be induced by chemical concentrations lower than those used in this work (Bérard and Benninghoff 2001, Debenest et al. 2010). Anthropogenic drivers such as toxic chemicals, global warming and overexploitation are causing large-scale shifts in the composition of primary producer and consumer communities, with concomitant impacts on the functioning of aquatic food webs (Rohr and Crumrine 2005, Relyea and Hoverman 2006, McIntyre et al. 2007, Moran et al. 2010, Hicks et al. 2011, McMahon et al. 2012, Litchman et al. 2015). Focusing not only on direct but also on compositional stress effects is thus encouraged to assess BEF relations under anthropogenic change.

6.5. Future perspectives

This doctoral thesis sets in many ways a baseline for the incorporation of anthropogenic stress into future research on biodiversity and ecosystem functioning research. The outcome of this work poses several challenges to further increase the level of realism in diversity-functioning research.

- A first challenge is to test which level of diversity is most relevant for ecosystem functioning under stress. In this work, stress consistently altered evenness, which was a better predictor of functioning than richness (but see 6.1), whilst most BEF experiments to date have focused on the functional role of species richness (Wittebolle et al. 2009, Cardinale et al. 2011, Tilman et al. 2014). Describing diversity at the species level however only stands in the middle of a broad diversity spectrum, which includes genetic diversity

(Hughes et al. 2008), functional trait diversity (Díaz et al. 2003, Cadotte et al. 2011) and the number of distinct communities within a system (Pasari et al. 2013).

    - To quantify which type of diversity is most relevant for ecosystem functioning under stress, future BEF research would need experiments which explicitly manipulate biodiversity at multiple levels. For example, is ecosystem functioning under stress driven by genetic diversity (e.g. through the presence of tolerant genotypes), trait diversity (e.g. the number of effect traits and their correlation with response traits), species diversity or community diversity (e.g. the number of distinct communities in a stressed system)?

- A second challenge is to include multiple anthropogenic stressors into BEF experiments. The few BEF experiments which included explicit exposure to anthropogenic stressors examined the impact of one type of stress in isolation (McMahon et al. 2012, Steudel et al. 2012, Radchuk et al. 2016, including this work). Ecosystems are however commonly exposed to multiple stressors (Lawler et al. 2006, Malaj et al. 2014).
    - Future BEF research will need approaches to predict community structure and ecosystem functioning in the presence of multiple anthropogenic stressors. Community ecotoxicology has developed advanced mechanistic and predictive approaches to predict biodiversity in the presence of multiple contaminants (Koelmans et al. 2001, Rohr et al. 2006, Clements and Rohr 2009, Halstead et al. 2014). Explicitly linking these multistressor approaches with the species' functional contribution represents a promising basis for testing the functional impact of multiple stressors, to further increase realism in BEF designs.
- A third challenge for future BEF research is to examine not only the functional consequences of species loss, but also of species gain. BEF research has been motivated by concerns about biodiversity loss, but global change, through species invasions and range expansions, is homogenizing the distribution of organisms worldwide (Cardinale et al. 2012, Dornelas et al. 2014). While global biodiversity indeed seems to decrease, biodiversity at local scales has been observed to remain unaffected or even increase (Sax and Gaines 2003, Dornelas et al. 2014, McGill et al. 2015).
    - In order to keep pace with a changing world, BEF research will need to develop designs that predict ecosystem functioning under simultaneous biodiversity losses and gains. The marine environment is largely affected by the homogenization of Earth's biota (Molnar et al. 2008, Vansteenbrugge et al. 2016), and coastal ecosystems are particularly vulnerable to invasive species (Carlton 1998, Gamfeldt et al. 2015). Marine studies could thus lead the way in integrating simultaneous invasion and extinction into BEF frameworks.

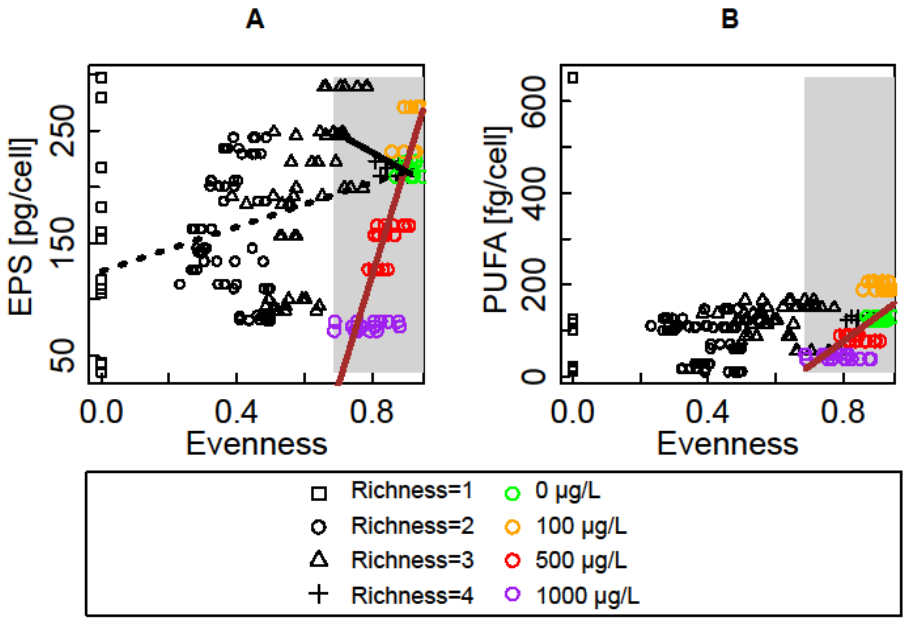
- Last, BEF research should further increase realism by including the spatial and temporal complexity driving the BEF relation in natural ecosystems. There still remains a large gap between the small spatial and temporal scales at which most BEF experiments are being performed, and those scales at which conservation efforts are taking place (Srivastava and Vellend 2005, Cardinale et al. 2011). Larger spatial and temporal scales allow for a greater niche partitioning, a key mechanism for complementarity (Cardinale et al. 2007, Stachowicz et al. 2008a, Venail et al. 2010). Facilitative interactions also grow stronger with time and can change with fluctuating abiotic conditions (Mulder et al. 2001, Cardinale et al. 2007, Stachowicz et al. 2008b).
    - This discrepancy of experimental and natural systems highlights the need to cross-check experimental results with observed patterns in natural ecosystems, and to transform the results of short-term laboratory or mesocosm BEF experiments into meaningful predictions at scales which matter to conservation. To extrapolate the results of controlled short-term experiments to more complex environments, BEF experiments should test biodiversity effects on functioning under constant conditions as well as different regimes of temporal and abiotic fluctuations. Once such an extrapolation degree has been established for various ecosystem processes, we should be able to transform the outcome of controlled small-scale BEF experiments to scales which are relevant for management and conservation efforts.

In a world affected by human activities at an increasing scale and speed, BEF research has come a long but fast way, from being a 'niche research area' at the interface of community and ecosystem ecology only twenty years ago to having a broad impact on both ecological research and public awareness. BEF research went through a contentious confirmatory phase in the 1990s and an exploratory phase in the 2000s revealing the core drivers of the biodiversity effect in functioning. Now, BEF research faces a realism phase with the challenge to include the natural and anthropogenic drivers of diversity-functioning relations into its experimental design. By complementing current BEF designs with the inherent variability of natural systems and the anthropogenic drivers of the ongoing biodiversity change, we should be able to gain a more thorough understanding of ecosystem functioning.

Addendum I – Supporting Material Chapter 2

Species	Shape	Biovolume equation	Length (a) [µm]	Width (b) [µm]	Height (c) [µm]	Biovolume [µm³]
Navicula arenaria	Elliptic prism	$V = \frac{\pi}{4} \times a \times b \times c$	47	18	15	**9896**
Entomoneis paludosa	Elliptic prism	$V = \frac{\pi}{4} \times a \times b \times c$	40	5	50	**7854**
Seminavis robusta	cymbelloid	$V = \frac{1}{6}\pi \times (2b)^2 \times a \times \frac{\beta}{360}$; with $sin\frac{\beta}{2} = \frac{c}{2 \times b}$	25	15	10	**2813**
Nitzschia sp.	Prism on parallelogram	$V = \frac{1}{2} \times a \times b \times c$	45	15	17	**5738**

Table S1: Cell biovolume per species. Biovolume was calculated based on the closest approximation of geometric shape and the mean measured linear dimensions during the experiments.



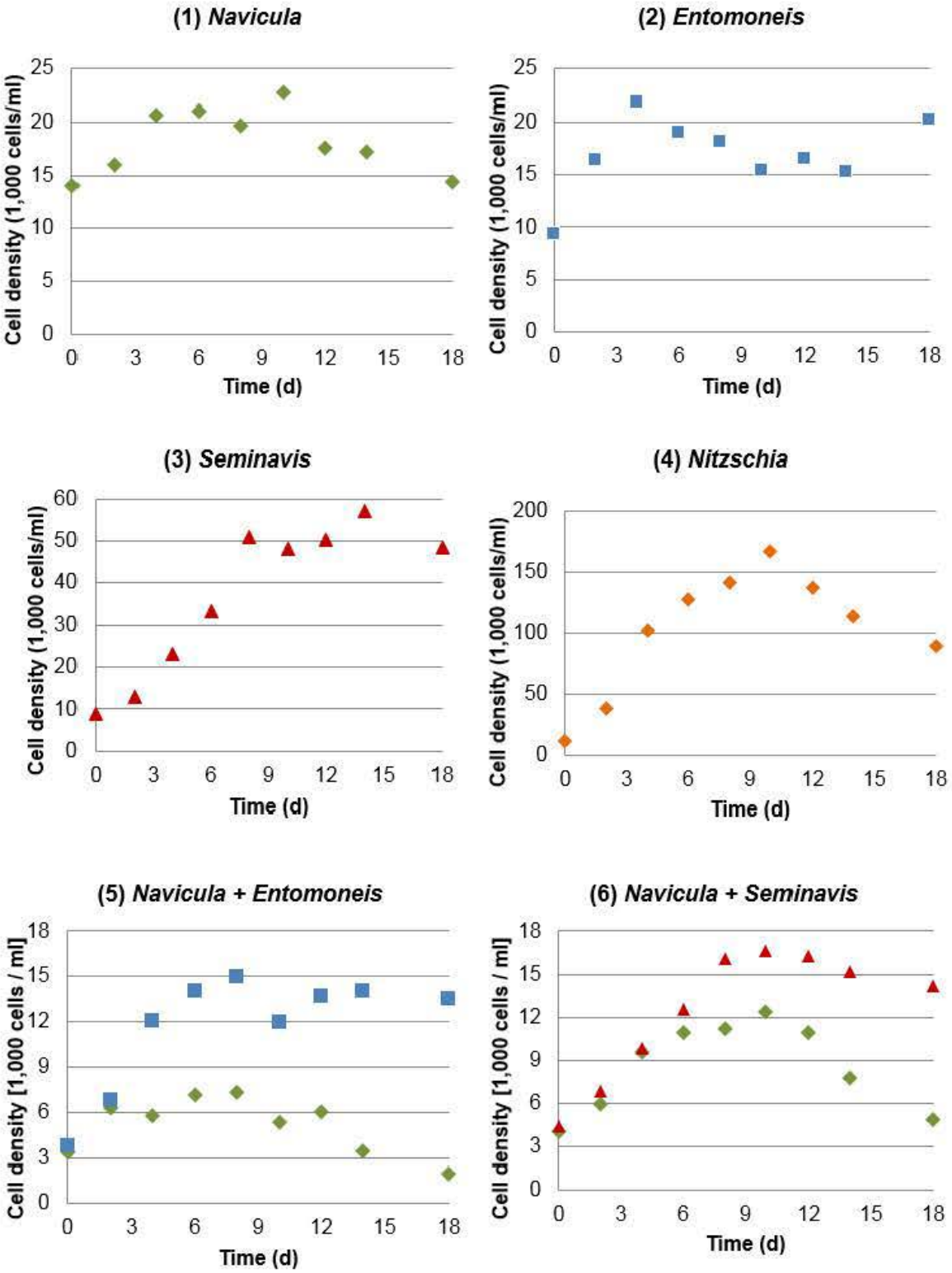
3833

3834 **Fig. S1:** EPS (A) and PUFA (B) concentrations normalized to cell density as a function of evenness.
3835 Regression lines show significant relations between evenness and function. Brown lines are linear models
3836 based on the atrazine experiment. Dashed and solid black lines are linear models based on the random
3837 assembly experiment for the whole evenness range or for the evenness range of the atrazine experiment
3838 (grey area), respectively.

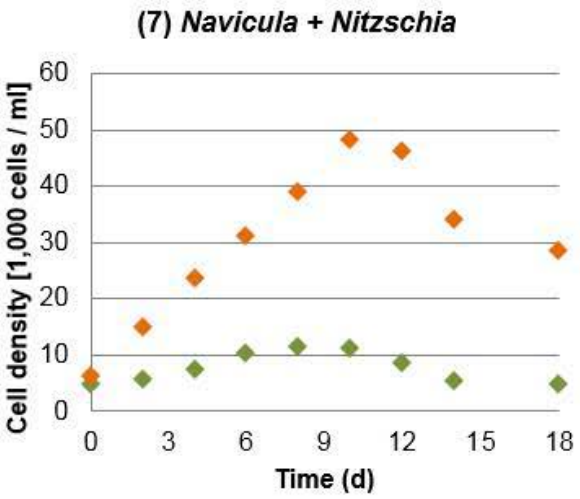
3839

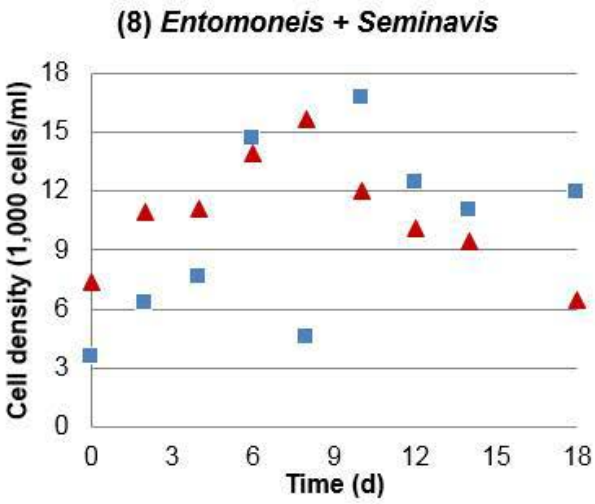
Ecosystem Function	Data	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Sediment stabilization: EPS [pg/ml]	R	1	78.22	18.45	4.24	**<0.0001**	2029	-1012	Yes		
		2	98.30	18.30	5.37	**<0.0001**	2023	-1008	Yes	7.78	**0.005**
	A	1	971.96	98.94	9.82	**<0.0001**	781	-387	Yes		
		2	894.93	83.61	10.70	**<0.0001**	780	-386	Yes	2.88	0.0897
	R‘	1	-188.23	81.60	-2.31	**0.0309**	234	-114	Yes		
		2	-166.25	64.94	-2.56	**0.0179**	221	-107	Yes	14.87	**0.0001**
Energy content: PUFA [fg/ml]	R	1	-25.99	31.09	-0.84	0.4043	2217	-1106	No		
		2	61.55	17.88	3.44	**0.0007**	2052	-1022	No	NA	NA
	A	1	663.37	74.62	8.89	**<0.0001**	740	-367	Yes		
		2	541.24	56.28	9.62	**<0.0001**	736	-364	Yes	5.80	**0.0160**
	R‘	1	-37.56	96.31	-0.39	0.7003	242	-118	No		
		2	63.13	63.25	1.00	0.3291	222	-107	No	NA	NA

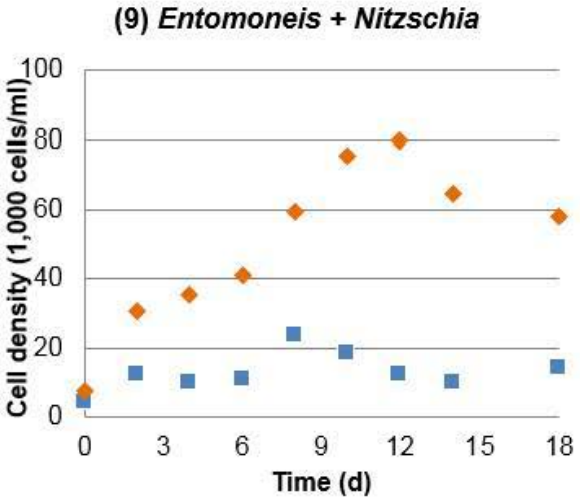
Table S2: Results of generalized least squared models predicting PUFA and EPS production (normalized to cell density) from evenness, without (model 1) or with (model 2) a variance structure. 'Data' denotes the data on which the models are based: the random assembly experiment (R), the atrazine experiment (A), the random assembly experiment for evenness values overlapping with those from the atrazine experiment (R'); 's.e.' is the standard error on the estimated slopes; T and P denote the t- and p-values, bold values are statistically significant. AIC is the Akaike information criterion; 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'); 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

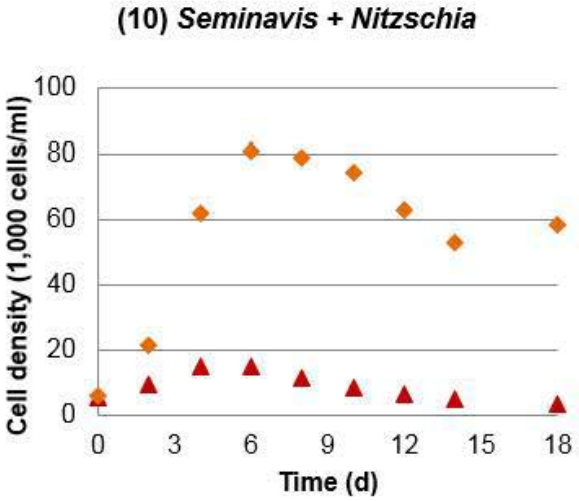

(1) Navicula
Cell density (1,000 cells/ml)
Time (d)
(2) Entomoneis
Cell density (1,000 cells/ml)
Time (d)
(3) Seminavis
Cell density (1,000 cells/ml)
Time (d)
(4) Nitzschia
Cell density (1,000 cells/ml)
Time (d)
(5) Navicula + Entomoneis
Cell density [1,000 cells / ml]
Time (d)
(6) Navicula + Seminavis
Cell density [1,000 cells / ml]
Time (d)

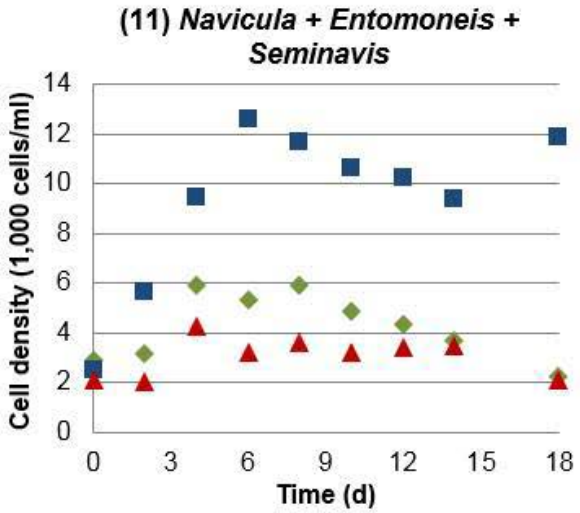
3850

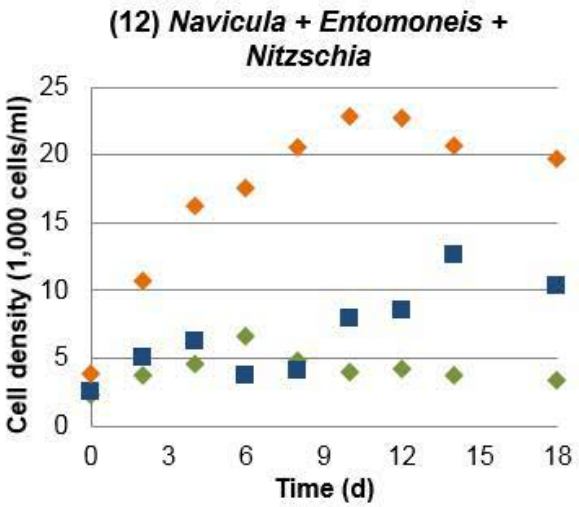

(7) Navicula + Nitzschia
Cell density [1,000 cells / ml]
Time (d)


(8) Entomoneis + Seminavis
Cell density (1,000 cells/ml)
Time (d)

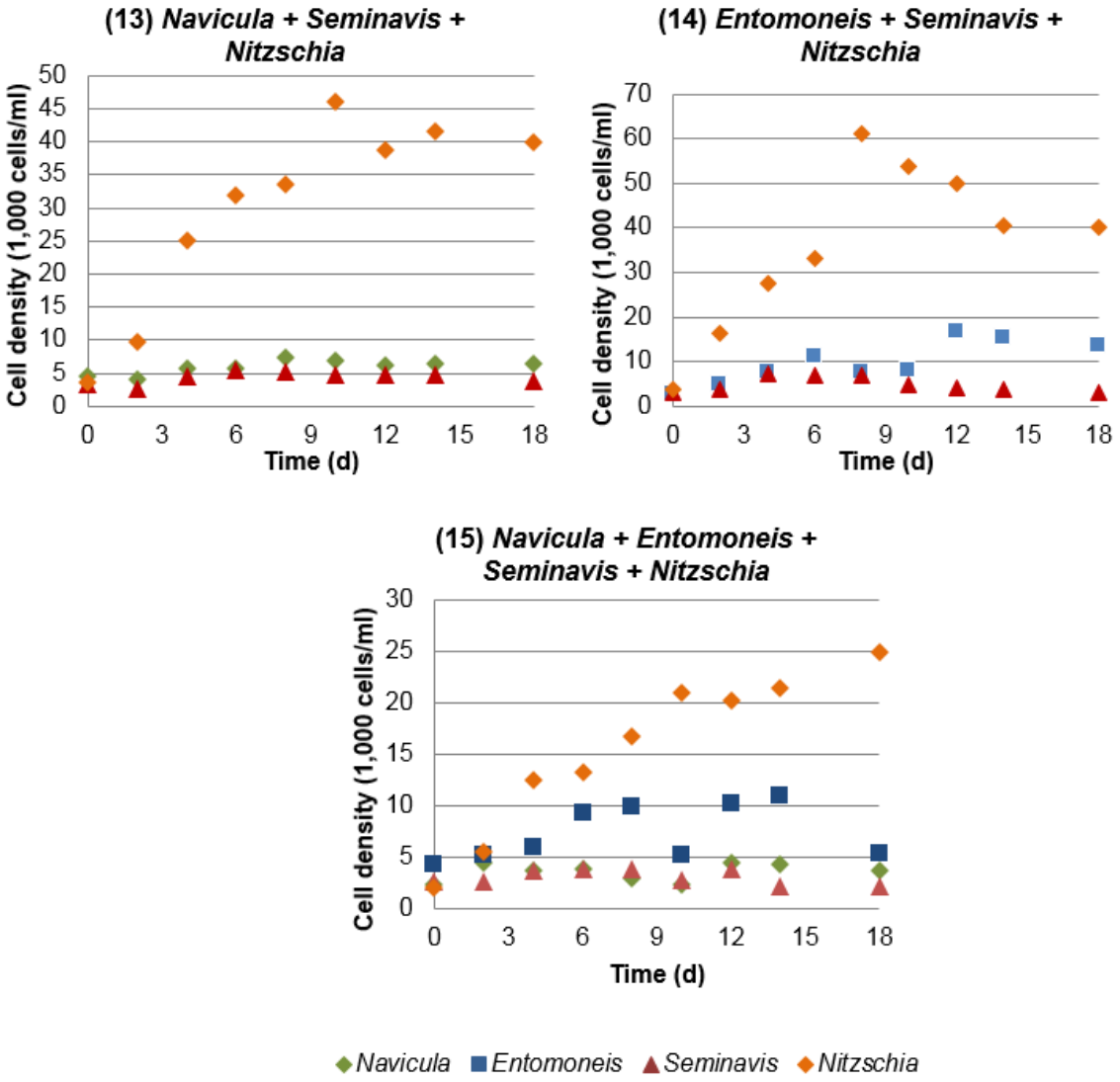

(9) Entomoneis + Nitzschia
Cell density (1,000 cells/ml)
Time (d)


(10) Seminavis + Nitzschia
Cell density (1,000 cells/ml)
Time (d)


(11) Navicula + Entomoneis + Seminavis
Cell density (1,000 cells/ml)
Time (d)


(12) Navicula + Entomoneis + Nitzschia
Cell density (1,000 cells/ml)
Time (d)

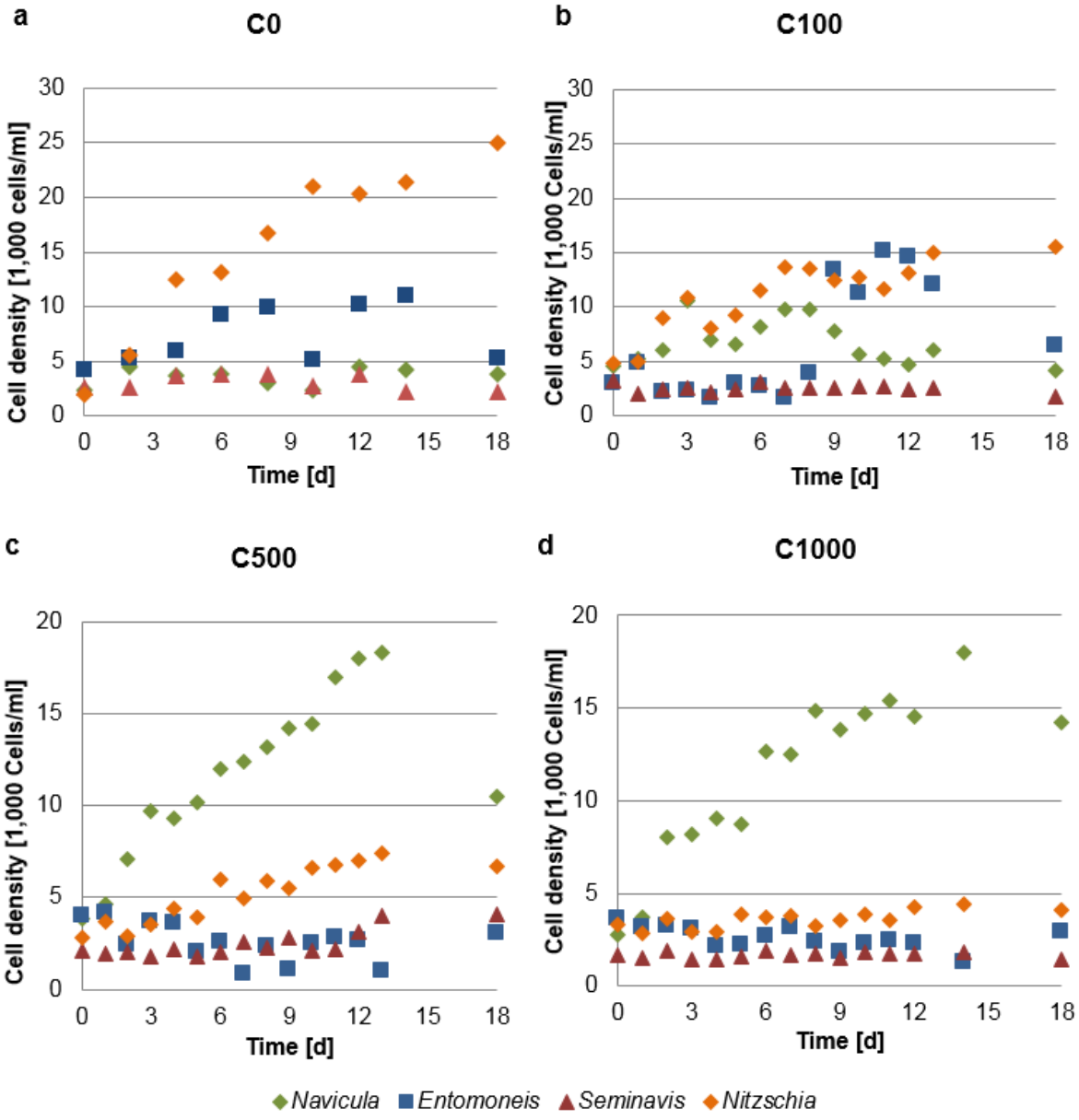
3851



3852

3853 **Fig. S2:** Cell density per species in the random assembly experiment**.** Treatments 1-15 (n=12) represent all
3854 possible random species combinations. Colour codes refer to the species *Navicula arenaria* (green),
3855 *Entomoneis paludosa* (blue), *Seminavis robusta* (red) and *Nitzschia sp.* (orange).

3856



3857

3858 **Fig. S3:** Cell density per diatom species in the atrazine experiment. Treatments C0, C100, C500 and C1000
3859 (0, 100, 500 and 1000 µg/l atrazine, n=18) are indicated in panels a, b, c and d respectively. Colour codes
3860 refer to the species *Navicula arenaria* (green), *Entomoneis paludosa* (blue), *Seminavis robusta* (brown) and
3861 *Nitzschia sp.* (orange).

3862

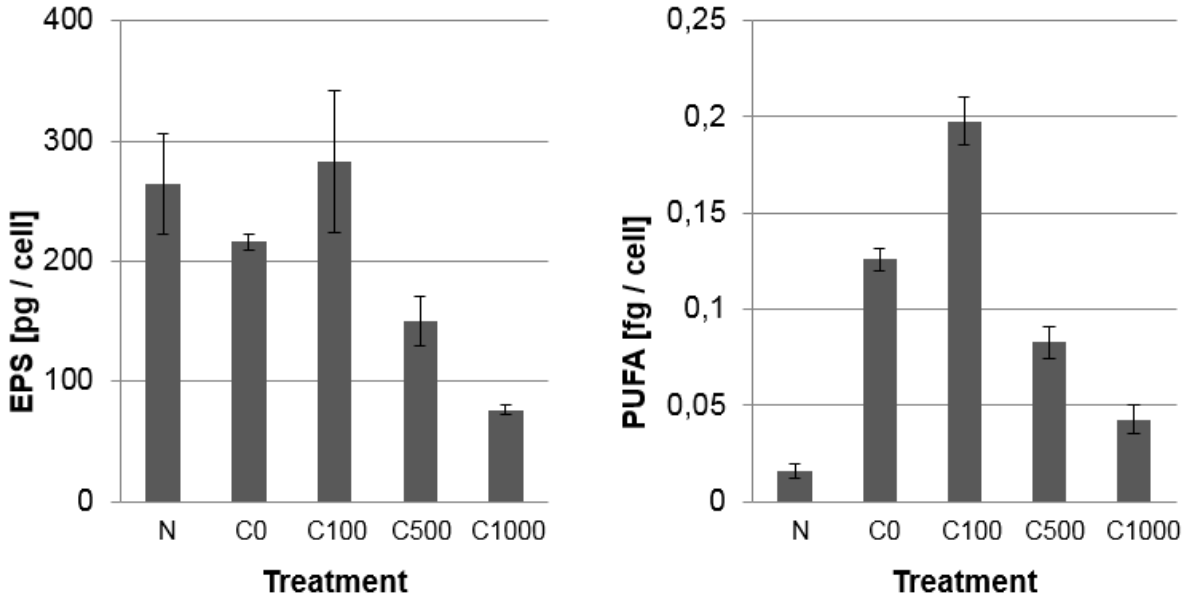


Fig. S4: EPS (a) and PUFA (b) concentrations normalized to cell density in monocultures of *Navicula arenaria* and atrazine-exposed communities. Treatments: N = *Navicula arenaria* monoculture, C0, C100, C500 and C1000 = full species community exposed to 0, 100, 500 and 1000 µg/l atrazine. Treatments C500 and C1000 were dominated by *Navicula arenaria* (s. Fig. S3). EPS: n=3, PUFA: n=2

Addendum II – Supporting Material Chapter 3

Species	Acc. Nr.	K [10^3 cells/ml]	Biovolume [µm3]	S:V	Length [µm]	Chl. a [F0]	TN [%]	TOC [%]	Mix 0.1	Mix 0,6	PE [Fv/Fm]	EPS total [mg/ml]	EPS / cell [ng/cell]	FA total [µg/ml]	FA / cell [pg/cell]
Cylindrotheca fusiformis	DCG 0423	997 (+- 32)	67 (+- 7)	1.57 (+- 0.08)	18.25 (+- 1.0)	0.34 (+- 0.05)	6.02 (+- 1.2)	41.67 (+- 0.3)	0.91 (+- 0.1)	1.00 (+- 0.1)	0.27 (+- 0.02)	1.32 (+- 0.01)	3.16 (+- 0.07)	931.10 (+- 310)	294.78 (+- 103)
***Nitzschia* sp. 1**	DCG 0483	170 (+- 26)	380 (+- 48)	0.61 (+- 0.04)	26.15 (+- 2.1)	0.27 (+- 0.09)	NA	17.71	0.89 (+- 0.2)	1.13 (+- 0.4)	0.46 (+- 0.02)	1.28 (+- 0.07)	16.56 (+- 2.86)	3.67 (+- 2.34)	58.84 (+- 23)
Nitzschia acicularis	DCG 0459	412 (+- 46)	517 (+- 68)	0.54 (+- 0.03)	33.01 (+- 2.5)	0.42 (+- 0.07)	7.26 (+- 0.6)	33.20 (+- 2.5)	1.22 (+- 0.1)	1.10 (+- 0.1)	0.56 (+- 0.02)	1.96 (+- 0.13)	9.99 (+- 0.32)	7.00 (+- 1.63)	39.24 (+- 8)
Amphora lineolata	DCG 0478	121 (+- 25)	778 (+- 123)	0.93 (+- 0.05)	26.39 (+- 1.5)	0.30 (+- 0.09)	9.03	43.84	0.18 (+- 0.1)	0.81 (+- 0.3)	0.64 (+- 0.04)	3.61 (+- 0.31)	89.09 (+- 0.72)	29.99 (+- 5.22)	767.20 (+- 228)
Cylindrotheca closterium		141 (+- 20)	345 (+- 60)	0.86 (+- 0.06)	25.46 (+- 1.0)	0.43 (+- 0.03)	5.28 (+- 1.7)	14.74 (+- 16)	2.36 (+- 0.3)	3.82 (+- 1.1)	0.53 (+- 0.04)	3.39 (+- 0.34)	67.02 (+- 4.48)	19.02 (+- 2.31)	313.14 (+- 35)
***Gyrosigma* sp. 2**	DCG 0491	42 (+- 6)	2081 (+- 241)	0.34 (+- 0.02)	44.58 (+- 2.2)	1.45 (+- 0.24)	7.41 (+- 2.8)	17.59 (+- 3.4)	1.87 (+- 0.5)	1.85 (+- 0.4)	0.64 (+- 0.01)	1.89 (+- 0.21)	157.19 (+- 25.46)	10.99 (+- 3.62)	820.91 (+- 207)
***Navicula arenaria* str. A2**	DCG 0487	58 (+- 11)	4466 (+- 388)	0.39 (+- 0.02)	39.49 (+- 2.8)	1.72 (+- 0.35)	2.86	22.19	0.16 (+- 0.1)	0.61 (+- 0.3)	0.52 (+- 0.04)	3.35 (+- 0.39)	279.30 (+- 15.80)	10.90 (+- 4.91)	782.41 (+- 155)
Astartiella bahusiensis	DCG 0469	86 (+- 28)	1552 (+- 239)	0.59 (+- 0.05)	33.19 (+- 0.3)	2.71 (+- 1.22)	7.49 (+- 2.9)	31.90 (+- 8.9)	0.89 (+- 0.2)	0.92 (+- 0.2)	0.63 (+- 0.02)	2.88 (+- 0.51)	84.10 (+- 9.75)	21.34 (+- 2.59)	687.92 (+- 41)
***Nitzschia* sp. 2**	DCG 0421	234 (+- 22)	650 (+- 46)	0.40 (+- 0.02)	23.69 (+- 1.4)	1.46 (+- 0.20)	6.51 (+- 0.2)	36.66 (+- 2.9)	0.98 (+- 0.3)	1.08 (+- 0.3)	0.65 (+- 0.01)	3.12 (+- 0.69)	76.48 (+- 15.01)	49.51 (+- 5.13)	895.53 (+- 182)
Gyrosigma sp. 1	DCG 0468	120 (+- 12)	2147 (+- 428)	0.35 (+- 0.04)	56.08 (+- 3.4)	1.14 (+- 0.19)	6.13 (+- 1.2)	32.29 (+- 1.4)	0.41 (+- 0.1)	0.90 (+- 0.3)	0.51 (+- 0.03)	0.57 (+- 0.24)	11.74 (+- 5.38)	12.22 (+- 4.79)	247.84 (+- 85)
***Navicula arenaria* str. A7**	DCG 0489	67 (+- 10)	3120 (+- 519)	0.46 (+- 0.04)	39.80 (+- 0.4)	1.62 (+- 0.37)	8.69 (+- 1.2)	25.19 (+- 6.4)	0.78 (+- 0.4)	0.38 (+- 0.2)	0.45 (+- 0.04)	2.39 (+- 0.13)	80.98 (+- 6.31)	8.95 (+- 1.41)	282.64 (+- 24)
Navicula digitoradiata	DCG 0490	38 (+- 6)	5738 (+- 640)	0.34 (+- 0.02)	35.50 (+- 1.0)	2.39 (+- 0.72)	13.90	17.71	1.40 (+- 0.5)	1.05 (+- 0.3)	0.49 (+- 0.01)	3.91 (+- 0.79)	254.63 (+- 51.01)	7.93 (+- 1.60)	508.04 (+- 46)

***Navicula* sp. 2**	DCG 0671	258 (+- 34)	587 (+- 113)	0.79 (+- 0.05)	21.46 (+- 1.6)	0.65 (+- 0.12)	8.10 (+- 0.1)	31.14 (+- 1.4)	0.79 (+- 0.3)	0.79 (+- 0.2)	0.37 (+- 0.03)	2.16 (+- 0.46)	17.31 (+- 3.41)	14.89 (+- 9.04)	144.67 (+- 87)
***Amphora* sp. 1**	DCG 0481	69 (+- 16)	533 (+- 87)	1.01 (+- 0.06)	24.21 (+- 1.3)	1.58 (+- 0.38)	11.45 (+- 2.9)	21.25 (+- 15)	0.75 (+- 0.2)	0.42 (+- 0.2)	0.58 (+- 0.05)	3.56 (+- 0.27)	158.77 (+- 29.13)	5.52 (+- 0.31)	215.01 (+- 58)
Biremis ambigua	DCG 0480	15 (+- 3)	4108 (+- 593)	0.37 (+- 0.02)	29.39 (+- 1.5)	2.99 (+- 0.66)	14.21	28.18	1.25 (+- 0.4)	1.50 (+- 0.7)	0.43 (+- 0.05)	1.51 (+- 0.12)	203.54 (+- 29.55)	2.24 (+- 0.41)	377.72 (+- 57)
***Entomoneis* sp.**	DCG 0466	45 (+- 12)	10932 (+- 2767)	0.28 (+- 0.03)	41.53 (+- 3.3)	2.97 (+- 0.71)	17.52 (+- 10)	22.79 (+- 9.9)	1.01 (+- 0.3)	0.96 (+- 0.2)	0.60 (+- 0.02)	0.59 (+- 0.03)	39.20 (+- 8.05)	20.78 (+- 3.27)	1292.26 (+- 489)
***Navicula* sp. 1**		302 (+- 46)	303 (+- 80)	1.02 (+- 0.12)	19.06 (+- 0.5)	1.11 (+- 0.24)	7.37 (+- 1.5)	26.21 (+- 1.9)	1.40 (+- 1.0)	1.54 (+- 0.6)	0.51 (+- 0.02)	2.19 (+- 0.49)	16.29 (+- 4.47)	8.86 (+- 7.91)	67.91 (+- 60)
***Amphora* sp. 2**	DCG 0672	132 (+- 7)	1052 (+- 183)	0.88 (+- 0.10)	24.57 (+- 1.2)	2.22 (+- 0.12)	7.97 (+- 0.5)	28.24 (+- 11)	1.09 (+- 0.2)	0.78 (+- 0.2)	0.56 (+- 0.03)	0.67 (+- 0.2)	11.03 (+- 3.35)	5.69 (+- 0.42)	78.96 (+- 7)

Table S1: Listing of the 18 diatom strains used in the experiments. From left to right are indicted: species name, strain accession number (Acc. Nr.) in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be), carrying capacity K (n=6), traits and functional contribution as determined in the control treatments of the atrazine experiment: biovolume (n=12), surface to volume ratio (S:V, n=12), length (n=12), chlorophyll a content (Chl. a, n=6; minimum chlorophyll fluorescence signal F_0 per 10^6 cells), % total nitrogen (TN, n=2 or 1), % total organic carbon (TOC, n=2 or 1), mixotrophy (Mix0.1 and Mix0.6, n=6, change of carrying capacity in the presence of 0.1 mM and 0.6 mM Glucose), photosynthetic efficiency (PE, n=6, ratio of variable to maximum fluorescence), EPS (n=3) per ml culture and per cell, polyunsaturated fatty acids (FA, n=2) per ml culture and per cell.

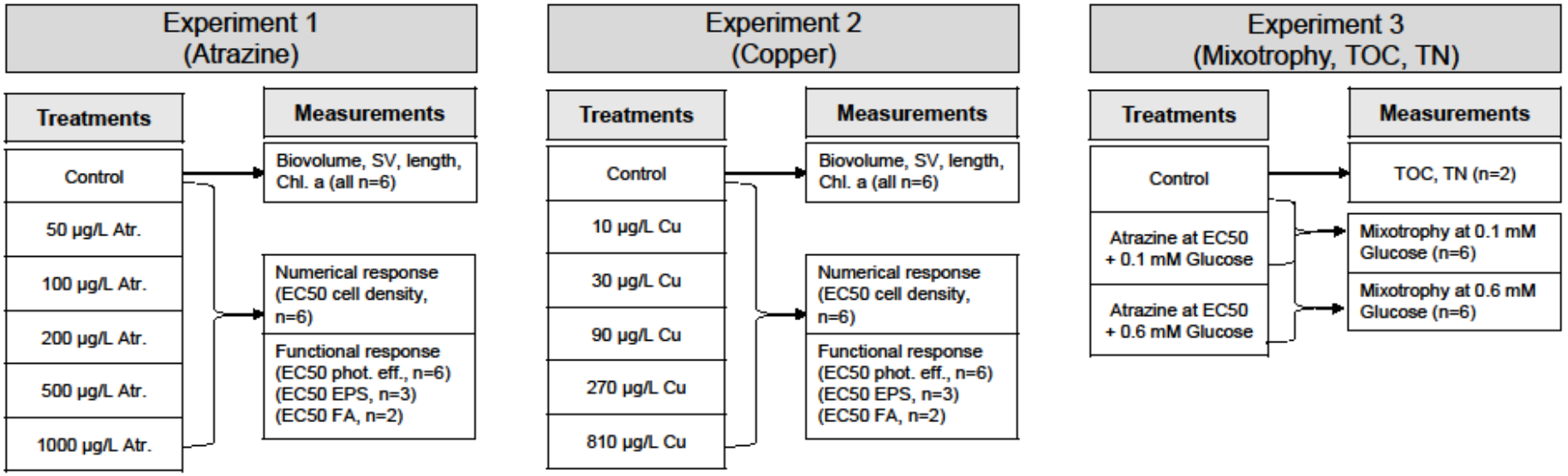


Fig. S2: Experimental setup. The following variables were measured: diatom biovolume, surface-to-volume ratio (SV), cell length, Chlorophyll a content (Chl. a), total organic carbon (TOC), total nitrogen (TN), the capacity for mixotrophic growth at 0.1 and 0.6 mM Glucose, and the copper and atrazine EC50 on cell density, photosynthetic efficiency (phot. eff), EPS and fatty acid (FA) production.

Substance	Theoretical concentration [µg/l]	Measured concentration [µg/l]
Control	0	<1
Atrazine	50	48
Atrazine	100	91
Atrazine	200	190
Atrazine	500	542
Atrazine	1000	1080
Copper	30	29
Copper	90	94
Copper	270	271
Copper	810	826

Table S3: Experimental atrazine and copper treatments with indication of theoretical and verified atrazine and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of quantification : < 1,0 µg/L atrazine and 2.6 µg/L copper for 10 ml samples).

	Biov	SV	Length	Chl. a	TN	TOC	Mix0.1	Mix0.6
Biov		0.58585	0.51675	0.71692	0.68926	0.30747	0.05789	0.17324
SV	0.58585		0.72220	0.53358	0.26675	0.37395	0.01088	0.08014
Length	0.51675	0.72220		0.37673	0.09360	0.23647	0.14848	0.14814
Chl. a	0.71692	0.53315	0.37673		0.52115	0.38792	0.12537	0.27885
TN	0.67866	0.27624	0.06867	0.50822		0.20132	0.09016	0.16944
TOC	0.30746	0.37395	0.23646	0.38792	0.13298		0.52557	0.39084
Mix0.1	0.05789	0.01088	0.14848	0.12537	0.09957	0.52557		0.83195
Mix0.6	0.17324	0.08014	0.14814	0.27885	0.16944	0.39084	0.83195	

Table S4a: Correlation matrix of traits to predict the numerical and functional response to atrazine. Biovolume (Biov), surface-to-volume ratio (SV), cell length, Chlorophyll a content (Chl. a) were measured in experiment 1. Total nitrogen (TN), total organic carbon (TOC) and mixotrophy (Mix0.1 and Mix0.6, n=6, change of maximum cell density in the presence of 0.1 mM and 0.6 mM Glucose) were measured in experiment 3. The correlation and subsequent analyses used the mean values of all traits.

	Biov	SV	Length	Chl. a	TN	TOC	Mix0.1	Mix0.6
Biov		0.584031	0.63121	0.61886	0.75572	0.32688	0.06539	0.19986
SV	0.58403		0.69247	0.49290	0.38780	0.29344	0.00895	0.11256
Length	0.63121	0.69247		0.24242	0.14788	0.27113	0.11168	0.14819
Chl. a	0.61886	0.49290	0.24242		0.74195	0.27100	0.00400	0.23797
TN	0.75572	0.38780	0.14788	0.74195		0.29626	0.07275	0.25834
TOC	0.32688	0.29344	0.27113	0.27100	0.29626		0.52424	0.39103
Mix0.1	0.06539	0.00895	0.11168	0.00400	0.07275	0.52424		0.82155
Mix0.6	0.19986	0.11256	0.14819	0.23797	0.25834	0.39103	0.82155	

Table S4a: Correlation matrix of traits to predict the numerical and functional response to copper. Biovolume (Biov), surface-to-volume ratio (SV), cell length, Chlorophyll a content (Chl. a) were measured in experiment 2. Total nitrogen (TN), total organic carbon (TOC) and mixotrophy (Mix0.1 and Mix0.6, n=6, change of maximum cell density in the presence of 0.1 mM and 0.6 mM Glucose) were measured in experiment 3. The correlation and subsequent analyses used the mean values of all traits.

Response	Data	Predictors	Slope	s.e.	T	P	AICc	Log lik.	R2	Adj. R2
Numerical: Carrying capacity	Cu	Biovolume	0.029	0.007	4.37	**0.0006**	205	-97	0.62	0.56
		Mix	-31.1	23.6	-1.32	0.21				
	Atr	Mix	133.8	33.2	4.03	**0.0012**	191	-89	0.62	0.56
		TOC	-2.13	3.00	-0.71	0.49				
Functional: Photosynthetic efficiency	Cu	Biovolume	0.12	0.03	3.25	*0.0059*	250	-118	0.57	0.47
		TOC	-6.35	12.9	-0.49	0.63				
		Mix	-143.9	136.6	-1.05	0.31				
	Atr	Biovolume	0.18	0.03	5.86	*0.0001*	233	-109	0.75	0.69
		TOC	4.22	11.22	0.38	0.71				
		Mix	210.2	119.7	1.76	0.10				
Functional: EPS	Cu	Biovolume	0.059	0.012	5.03	**0.0002**	197	-93	0.71	0.67
		Mix	-57.2	42.1	-1.36	0.20				
	Atr	TOC	-4.20	3.05	-1.38	0.19	191	-89	0.23	0.12
		Mix	-64.9	33.6	-1.93	0.08				
Functional: Fatty acids	Cu	Biovolume	0.042	0.006	6.69	**<0.0001**	168	-78	0.82	0.79
		Mix	-11.1	19.8	-0.56	0.58				
	Atr	Biovolume	0.027	0.010	2.85	*0.0128*	205	-97	0.41	0.33
		Mix	-26.2	34.6	-0.76	0.46				

Table S5: Results of generalized least squares models predicting copper and atrazine EC50 on diatom cell density (numerical response) and copper and atrazine EC50 on diatom effect traits (functional response) from traits; 'Response' denotes the response variable: numerical or functional response in terms of

photosynthetic efficiency, EPS or fatty acid production; 'Data' denotes the data on which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); Model selection was performed with a backward stepwise elimination of predictors, with evaluation of model fit by AIC and log likelihood. Predictors in the best-fitting models are biovolume, total organic carbon (TOC) and mixotrophic response to 0.6 mM Glucose concentrations (Mix); 'Slope' indicates the relation between predictors and numerical and functional response to copper and atrazine; 's.e.' is the standard error on the estimated slopes; 'T' and 'P' denote the t- and p-values, bold values are statistically significant, italic values are statistically significant over the whole dataset but not if outlier species are omitted (model fits without outlier species are then indicated in Table 2); 'AICc' is the Akaike information criterion with a correction for finite sample sizes, 'Log lik' the log-likelihood ratio. Only best-fitting models with uncorrelated predictors are shown (correlation factor < 0.5); Additional model fits with traits that also predicted numerical and functional response to copper and atrazine, but were correlated to biovolume and mixotrophy (correlation factor > 0.5), are indicated in Table 2. 'R2' and 'Adj. R2' are likelihood-ratio based pseudo-R-squared and adjusted R-squared values.

Response	Data	Predictors	Slope	s.e.	T	P	AICc	Log lik.	R2	Adj. R2
Numerical: Carrying capacity	Cu‘	Biovolume	0.038	0.011	3.36	**0.0047**	193	-91	0.53	0.46
		Mix	-27.2	24.0	-1.13	0.28				
	Cu	SV	-185.2	84.1	-2.20	**0.0463**	179	-82	0.75	0.69
		TN	8.12	7.34	1.11	0.29				
		Mix	-35.3	29.0	-1.22	0.25				
		Length	6.76	1.32	5.13	**0.0002**	172	-73	0.89	0.86
		Chl. a	-16.7	25.0	-0.67	0.52				
		TN	14.7	6.7	2.18	*0.0499*				
		Mix	-25.4	19.9	-1.27	0.23				
	Atr‘	Mix	161.7	64.1	2.52	**0.0348**	178	-83	0.34	0.24
		TOC	-2.22	3.11	-0.71	0.49				
Functional: Photosynthetic efficiency	Cu	Length	24.6	9.0	2.75	**0.0189**	214	-94	0.85	0.78
		Chl. a	-227.9	167.3	-1.36	0.20				
		TOC	-7.74	16.5	-0.47	0.65				
		TN	96.46	42.2	2.28	*0.0434*				
		Mix	-141.6	160.8	-0.88	0.40				
	Cu‘	Biovolume	0.08	0.07	1.21	0.25	235	-110	0.32	0.17
		TOC	-9.85	14.1	-0.70	0.50				
		Mix	-177	147.2	-1.21	0.25				
	Atr“	Biovolume	0.07	0.04	1.85	0.09	210	-97	0.41	0.26
		TOC	-4.38	8.33	-0.53	0.61				
		Mix	133.0	87.9	1.51	0.15				
Functional:	Cu	Length	11.0	3.56	3.08	**0.0104**	175	-79	0.85	0.81

EPS		Chl. a	-63.2	62.9	-1.00	0.34				
		TN	40.9	17.1	2.39	*0.0359*				
	Cu‘	Biovolume	0.060	0.021	2.82	**0.0156**	185	-87	0.52	0.46
		Mix	-56.6	45.2	-1.25	0.23				
Functional: Fatty acids	Cu	SV	-305	69.5	-4.39	**0.0017**	139	-58	0.94	0.92
		TOC	-1.14	2.76	-0.41	0.69				
		TN	28.5	6.8	4.20	*0.0023*				
		Mix	8.92	27.0	0.33	0.75				
		Length	5.67	1.86	3.04	**0.0141**	146	-61	0.90	0.87
		Chl. a	11.2	34.8	0.32	0.76				
		TN	26.5	9.47	2.79	*0.0209*				
		Mix	9.30	27.5	0.34	0.74				
	Cu‘	Biovolume	0.046	0.011	3.74	**0.0032**	157	-172	0.61	0.55
		Mix	-8.86	21.2	-0.42	0.68				
	Atr’’	Biovolume	0.004	0.015	0.30	0.77	190	-89	0.10	-0.03
		Mix	-33.5	31.6	-1.06	0.31				

Table S6: Results of generalized least squares models predicting copper and atrazine EC50 on diatom cell density (numerical response) and copper and atrazine EC50 on diatom effect traits (functional response) from traits; 'Response' denotes the response variable: numerical and functional response in terms of photosynthetic efficiency, EPS or fatty acid production; 'Data' denotes the data on which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); the copper experiment without the largest species (*Entomoneis* sp., (Cu')), the atrazine experiment without the most mixotrophic species (*Cylindrotheca closterium*, (Atr')), and the atrazine experiment without the largest species (*Entomoneis* sp., (Atr'')); Model selection was performed with a backward stepwise elimination of predictors, with evaluation of model fit by AIC and log likelihood. Predictors in the best-fitting models are biovolume, surface-to-volume ratio (SV), Chlorophyll a content (Chl. a), Length, total nitrogen (TN), total organic carbon (TOC) and mixotrophic response to 0.6 mM Glucose concentrations (Mix); 'Slope' indicates the relation between predictors and numerical and functional response to copper and atrazine; 's.e.' is the standard error on the estimated slopes; Only models with uncorrelated predictors that predicted numerical and functional response are shown ($p<0.05$, correlation factor <0.5); 'T' and 'P' denote the t- and p-values, bold values are statistically significant, italic values are statistically significant over the whole dataset but not if outlier species are omitted; 'AICc' is the Akaike information criterion with a correction for finite sample sizes, 'Log lik' the log-likelihood. ‘R2’ and ‘Adj. R2’ are likelihood-ratio based pseudo-R-squared and adjusted R-squared values.

FR	Data	Mod	Predictor	Slope	s.e.	T	P	AIC	Log lik.	R2	Adj. R2
Prim. Production: Phot. efficiency	Cu	B	EC50 K	3.20	0.30	10.51	**<0.0001**	1535	-763	0.68	0.68
	Atr	B	EC50 K	0.89	0.37	2.41	**0.0175**	1579	-786	0.12	0.12
Sed. stabilization: EPS	Cu	A	EC50 K	1.26	0.23	5.50	**<0.0001**	630	-312	0.40	0.39
	Atr	B	EC50 K	-0.21	0.04	-5.44	**<0.0001**	620	-306	0.38	0.36
Energy content: Fatty acids	Cu	B	EC50 K	0.67	0.19	3.52	**0.0015**	360	-176	0.54	0.52
	Atr	B	EC50 K	-0.26	0.11	-2.32	**0.0266**	429	-211	0.13	0.10

Table S7: Results of generalized least squares models relating copper and atrazine EC50 on diatom cell density (numerical response) and copper and atrazine EC50 on diatom effect traits (functional response). 'FR' denotes the response variable: functional response to copper or atrazine in terms of photosynthetic efficiency, fatty acid or EPS production; 'Data' denotes the data on which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); 'Mod' indicates if the model was fitted without (Model A) or with (Model B) variance structure allowing residuals to change with the predictor; Predictors are the numerical response to copper and atrazine respectively; 'Slope' indicates the relation between numerical and functional response; 's.e.' is the standard error on the estimated slopes; 'T' and 'P' denote the t- and p-values, bold values are statistically significant; 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'R2' and 'Adj. R2' are likelihood-ratio based pseudo-R-squared values.

Species	EC50 [µg/l]			
	Carrying capacity	Photosynthetic efficiency	EPS / cell	Fatty acids / cell
Cylindrotheca fusiformis	217.19 (3.15) *54.94 (10.01)*	221.29 (13.46) *75.77 (27.98)*	220.05 (101.35) *40.91 (8.03)*	23.62 (21.22)
Nitzschia sp. 1	137.09 (40.09) *96.46 (22.94)*	231.41 (42.18) *712.15 (65.91)*	478.08 (229.89) *157.14 (56.86)*	95.71 (3.90) *84.03 (7.80)*
Nitzschia acicularis	213.31 (33.58) *281.12 (44.41)*	292.72 (23.14) *1511.18 (299.76)*	112.91 (10.87) *531.48 (172.67)*	236.54 (11.01) *117.07 (79.21)*
Amphora lineolata	175.08 (18.32) *287.76 (131.17)*	391.50 (44.71) *703.83 (98.68)*	192.31 (157.25) *178.38 (47.59)*	66.54 (2.04) *107.58 (18.31)*
Cylindrotheca closterium	652.28 (171.90) *39.14 (10.50)*	987.78 (65.51) *256.40 (54.26)*	59.59 (7.64) *31.19 (0.94)*	4.78 (3.33) *13.71 (7.75)*
Gyrosigma sp. 2	453.41 (159.01) *271.42 (98.76)*	499.60 (32.72) *730.73 (15.75)*	216.74 (88.37)	85.50 (79.62) *325.09 (115.22)*
Navicula arenaria str. A2	349.01 (128.63) *219.22 (50.81)*	1144.84 (257.17) *1005.53 (325.52)*	176.89 (16.70) *581.77 (41.40)*	209.07 (57.34) *179.27 (1.95)*
Astartiella bahusiensis	328.79 (84.83) *243.28 (54.76)*	296.67 (26.56) *479.63 (118.04)*	172.73 (27.45) *158.30 (35.39)*	325.68 (18.47) *163.35 (137.67)*
Nitzschia sp. 2	161.77 (49.61) *64.78 (17.83)*	292.78 (34.05) *434.83 (78.93)*	76.94 (57.10) *237.49 (54.50)*	47.63 (29.93) *89.58 (32.25)*
Gyrosigma sp. 1	337.01 (72.29) *180.63 (58.23)*	759.70 (41.81) *403.43 (22.28)*	253.62 (154.76) *175.52 (42.58)*	134.51 (26.60) *95.28 (3.14)*
Navicula arenaria str. A7	171.36 (32.67) *348.17 (152.21)*	275.68 (42.65) *1267.94 (370.70)*	379.90 (54.36) *515.57 (197.42)*	68.40 (5.79) *288.86 (4.92)*
Navicula digitoradiata	217.51 (120.77) *258.05 (87.62)*	616.91 (77.63) *955.84 (102.73)*	184.35 (64.45) *380.21 (230.53)*	138.98 (57.13)
Navicula sp. 2	57.61 (16.99) *70.01 (26.09)*	446.60 (89.33) *157.66 (18.38)*	189.20 (12.23) *258.57 (104.69)*	52.52 (66.30) *189.24 (1.36)*
Amphora sp. 1	230.13 (124.85) *132.97 (46.56)*	551.16 (182.05) *698.06 (29.55)*	113.50 (42.56) *190.75 (85.02)*	205.04 (0.12) *80.79 (91.09)*
Biremis ambigua	415.03 (76.71) *134.41 (32.92)*	581.31 (96.54) *144.12 (27.81)*	178.98 (85.62) *205.49 (85.05)*	119.23 (22.55)
Entomoneis sp.	329.94 (230.12) *396.63 (61.51)*	2676.35 (746.98) *1934.08 (488.57)*	251.21 (98.69) *792.79 (75.41)*	507.03 (185.09) *502.39 (243.05)*
Navicula sp. 1	400.87 (101.04) *35.12 (12.05)*	404.32 (48.76) *175.19 (13.18)*	155.33 (28.56) *140.05 (33.51)*	262.52 (231.74) *3.36 (0.75)*
Amphora sp. 2	*148.73 (15.07)*	*626.61 (33.71)*		*100.27 (9.66)*
Average	288.32 (143.79) *181.27 (111.43)*	627.70 (589.20) *631.83 (504.96)*	200.71 (103.06) *299.87 (212.88)*	150.72 (129.87) *141.89 (124.90)*

Table S8: Atrazine and copper EC50s obtained for carrying capacity (n=6), photosynthetic efficiency (n=6), fatty acid (n=2) and EPS production (n=3) of 18 benthic diatom strains. The first line refers to the results with atrazine, the second with copper (italics). Standard deviations are indicated in brackets.

Species	Biovolume Control		Biovolume Atrazine		T	P
Cylindrotheca fusiformis	68	(+- 20)	84	(+- 23)	-1.87	0.08
Nitzschia sp. 1	380	(+- 81)	406	(+- 102)	-0.14	0.88
Nitzschia acicularis	514	(+- 80)	508	(+- 127)	0.13	0.90
Amphora lineolata	527	(+- 91)	553	(+- 113)	-0.64	0.53
Cylindrotheca sp. 2	340	(+- 94)	281	(+- 64)	1.78	0.09
Gyrosigma sp. 2	2078	(+- 252)	2013	(+- 179)	0.73	0.47
Navicula arenaria A2	4447	(+- 670)	4510	(+- 687)	-0.23	0.82
Astartiella bahusiensis	1540	(+- 303)	1794	(+- 832)	-0.99	0.34
Nitzschia sp. 2	643	(+- 99)	593	(+- 171)	0.88	0.39
Gyrosigma sp. 1	2134	(+- 458)	2134	(+- 411)	<0.01	0.99
Navicula arenaria A7	3131	(+- 717)	3074	(+- 427)	0.23	0.82
Navicula digitoradiata	5753	(+- 694)	5316	(+- 762)	1.47	0.16
Navicula sp. 2	568	(+- 102)	585	(+- 145)	-0.33	0.75
Amphora sp. 1	527	(+- 91)	553	(+- 113)	-0.64	0.53
Biremis ambigua	4082	(+- 575)	3872	(+- 773)	0.76	0.46
Entomoneis sp.	11124	(+- 2654)	11976	(+- 2108)	-0.87	0.39
Navicula sp. 1	304	(+- 80)	342	(+- 50)	-1.41	0.18

Table S9 a: Biovolume per species at the end of the experiment, in control and atrazine (500 µg/L) treatments. T and P denote the t- and p-values from a two-sided t-test testing differences in biovolume in control vs. atrazine treatments, bold values are statistically significant (p<0.05).

Species	Biovolume Control		Biovolume Cu		T	P
Cylindrotheca fusiformis	149	(+- 57)	122	(+- 44)	-1.30	0.21
Nitzschia sp. 1	366	(+- 95)	438	(+- 88)	-1.79	0.09
Nitzschia acicularis	622	(+- 144)	712	(+- 189)	-1.31	0.20
Amphora lineolata	967	(+- 247)	1080	(+- 213)	-1.20	0.25
Cylindrotheca sp. 2	334	(+- 64)	342	(+- 127)	-0.20	0.84
Gyrosigma sp. 2	2873	(+- 767)	2522	(+- 613)	1.24	0.23
Navicula arenaria A2	4214	(+- 723)	4139	(+- 672)	0.26	0.79
Astartiella bahusiensis	1418	(+- 242)	1779	(+- 140)	-4.48	**0.0003**
Nitzschia sp. 2	612	(+- 141)	702	(+- 160)	-1.45	0.16
Gyrosigma sp. 1	1722	(+- 509)	1782	(+- 454)	-0.30	0.76
Navicula arenaria A7	5077	(+- 1204)	4146	(+- 828)	1.45	0.17
Navicula digitoradiata	5698	(+- 1155)	5359	(+- 1015)	0.76	0.18
Navicula sp. 2	884	(+- 96)	780	(+- 154)	1.98	0.06
Amphora sp. 1	739	(+- 147)	740	(+- 114)	-0.02	0.98
Biremis ambigua	2650	(+- 482)	2843	(+- 707)	-0.78	0.45
Entomoneis sp.	10621	(+- 2743)	11607	(+- 1786)	-1.04	0.31
Navicula sp. 1	270	(+- 43)	313	(+- 93)	-1.45	0.17
Cylindrotheca fusiformis	739	(+- 147)	740	(+- 114)	-0.02	0.98

Table S9 b: Biovolume per species at the end of the experiment, in control and copper (270 µg/L) treatments. T and P denote the t- and p-values from a two-sided t-test testing differences in biovolume in control vs. atrazine treatments, bold values are statistically significant ($p<0.05$).

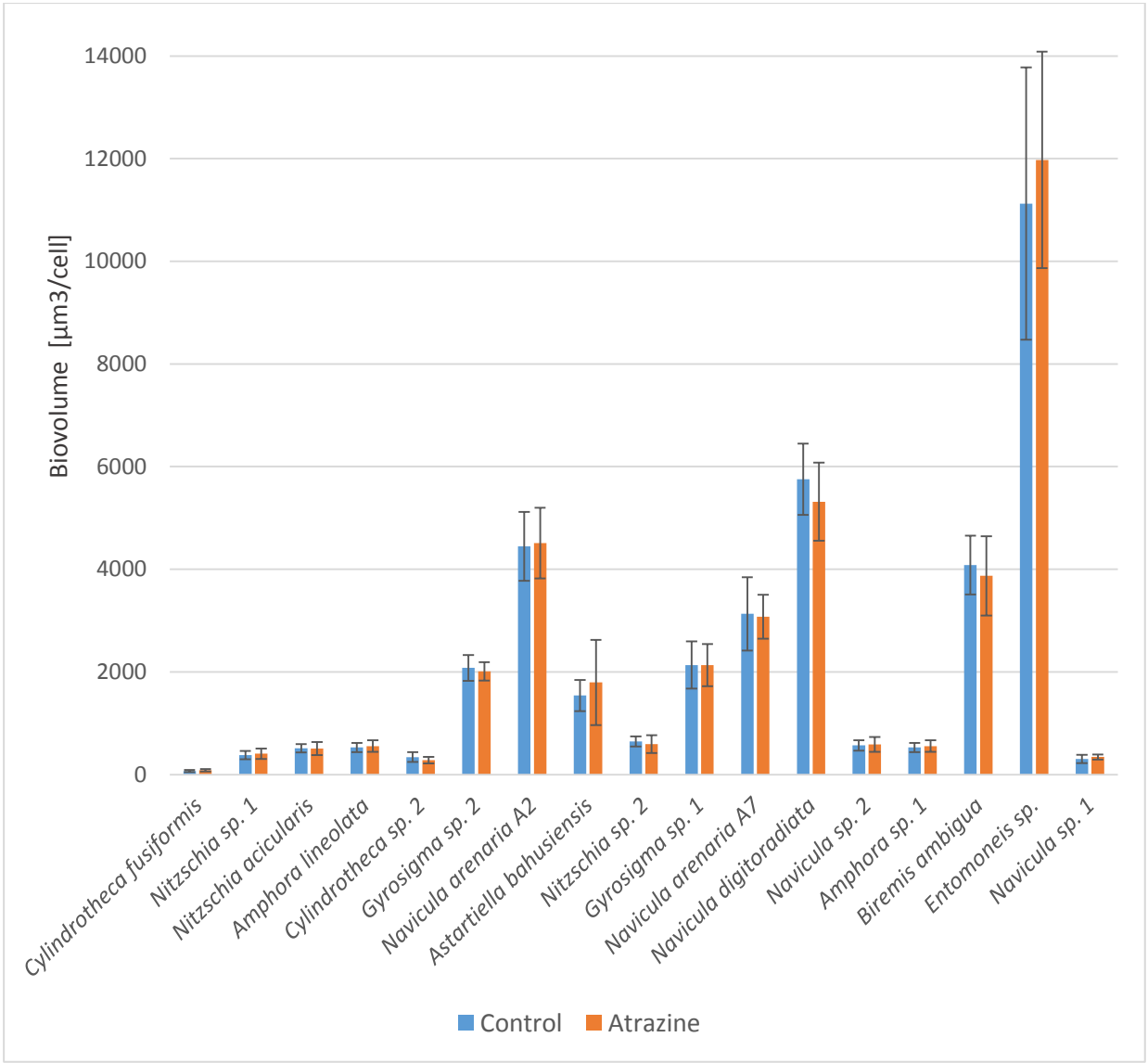


Fig. S9 a: Average biovolume per species in control (blue bars) and atrazine (500 µg/L, red bars) treatments

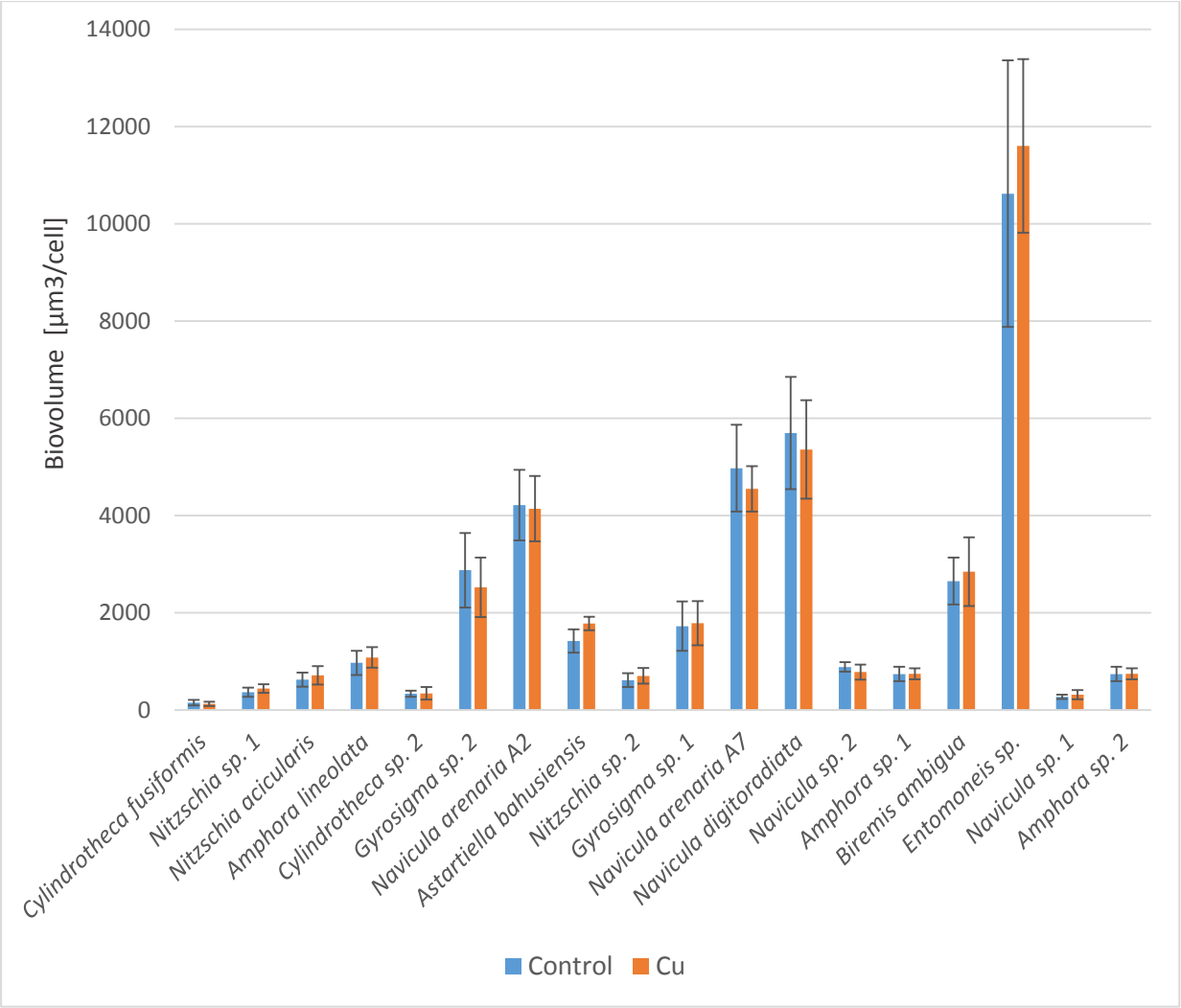
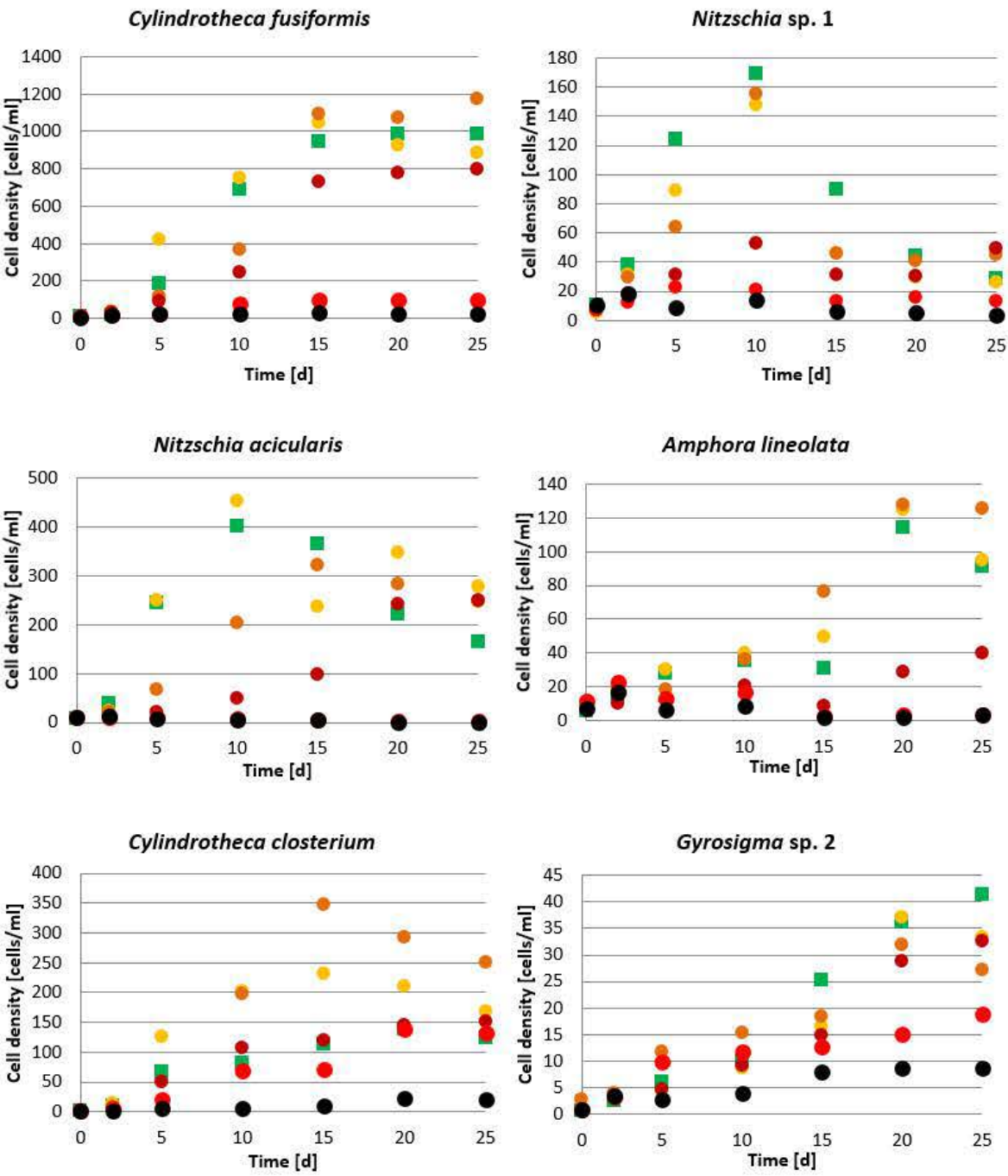
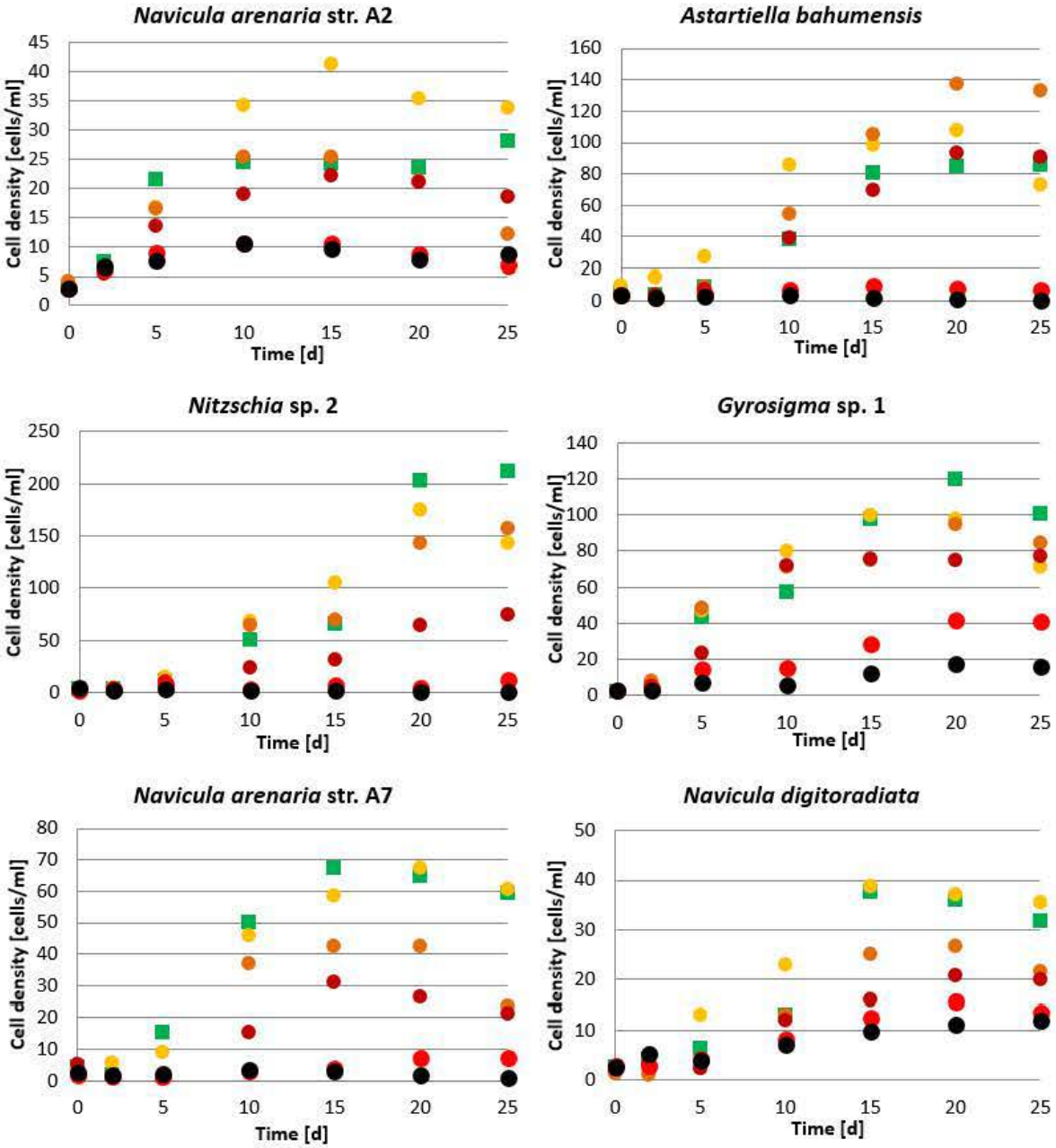


Fig. S9 b. Average biovolume per species in control (blue bars) and copper (270 µg/L, red bars) treatments


Cylindrotheca fusiformis
Cell density [cells/ml]
Time [d]
Nitzschia sp. 1
Cell density [cells/ml]
Time [d]
Nitzschia acicularis
Cell density [cells/ml]
Time [d]
Amphora lineolata
Cell density [cells/ml]
Time [d]
Cylindrotheca closterium
Cell density [cells/ml]
Time [d]
Gyrosigma sp. 2
Cell density [cells/ml]
Time [d]

3981


Navicula arenaria str. A2
Cell density [cells/ml]
Time [d]
Astartiella bahumensis
Cell density [cells/ml]
Time [d]
Nitzschia sp. 2
Cell density [cells/ml]
Time [d]
Gyrosigma sp. 1
Cell density [cells/ml]
Time [d]
Navicula arenaria str. A7
Cell density [cells/ml]
Time [d]
Navicula digitoradiata
Cell density [cells/ml]
Time [d]

3982

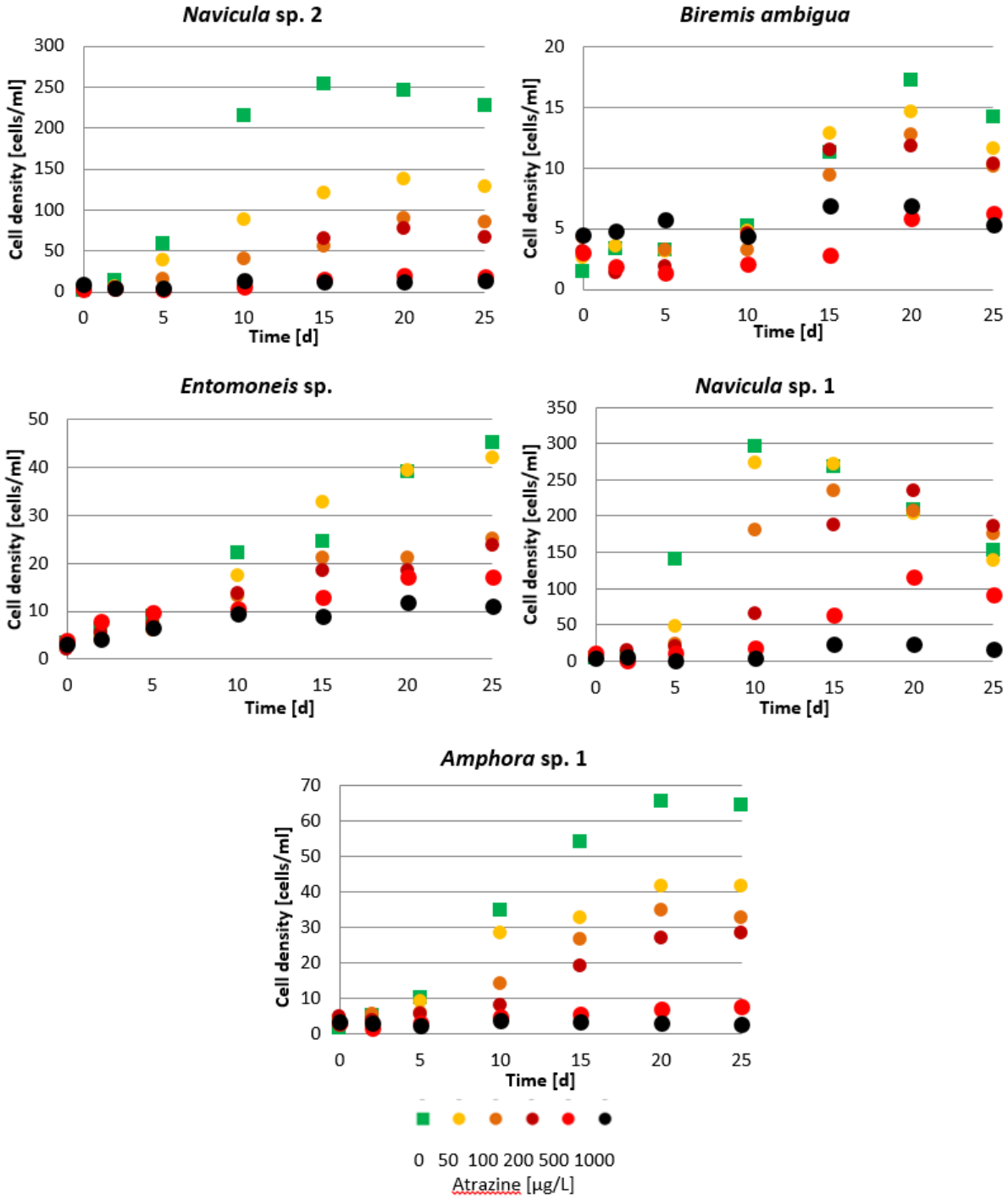
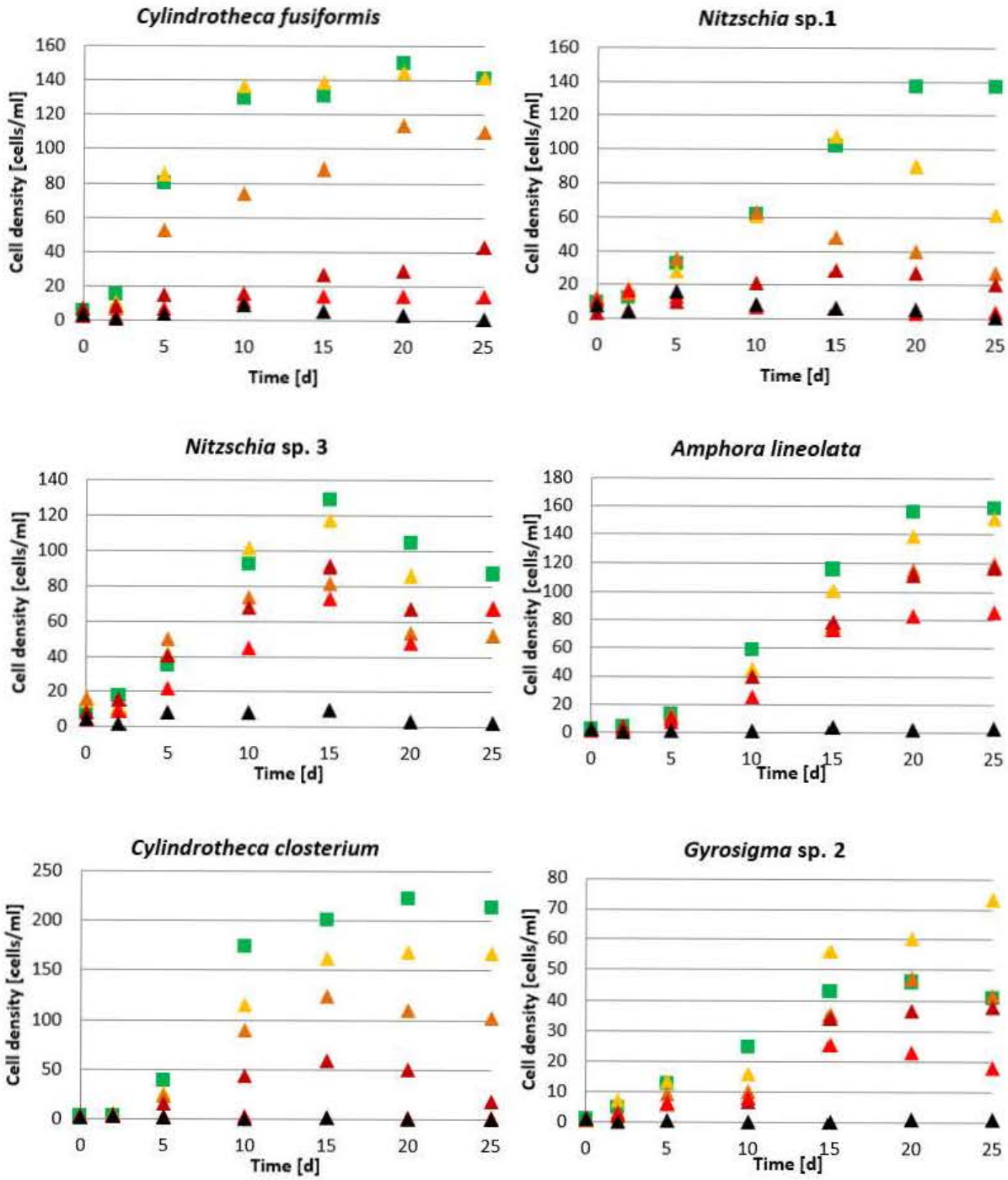
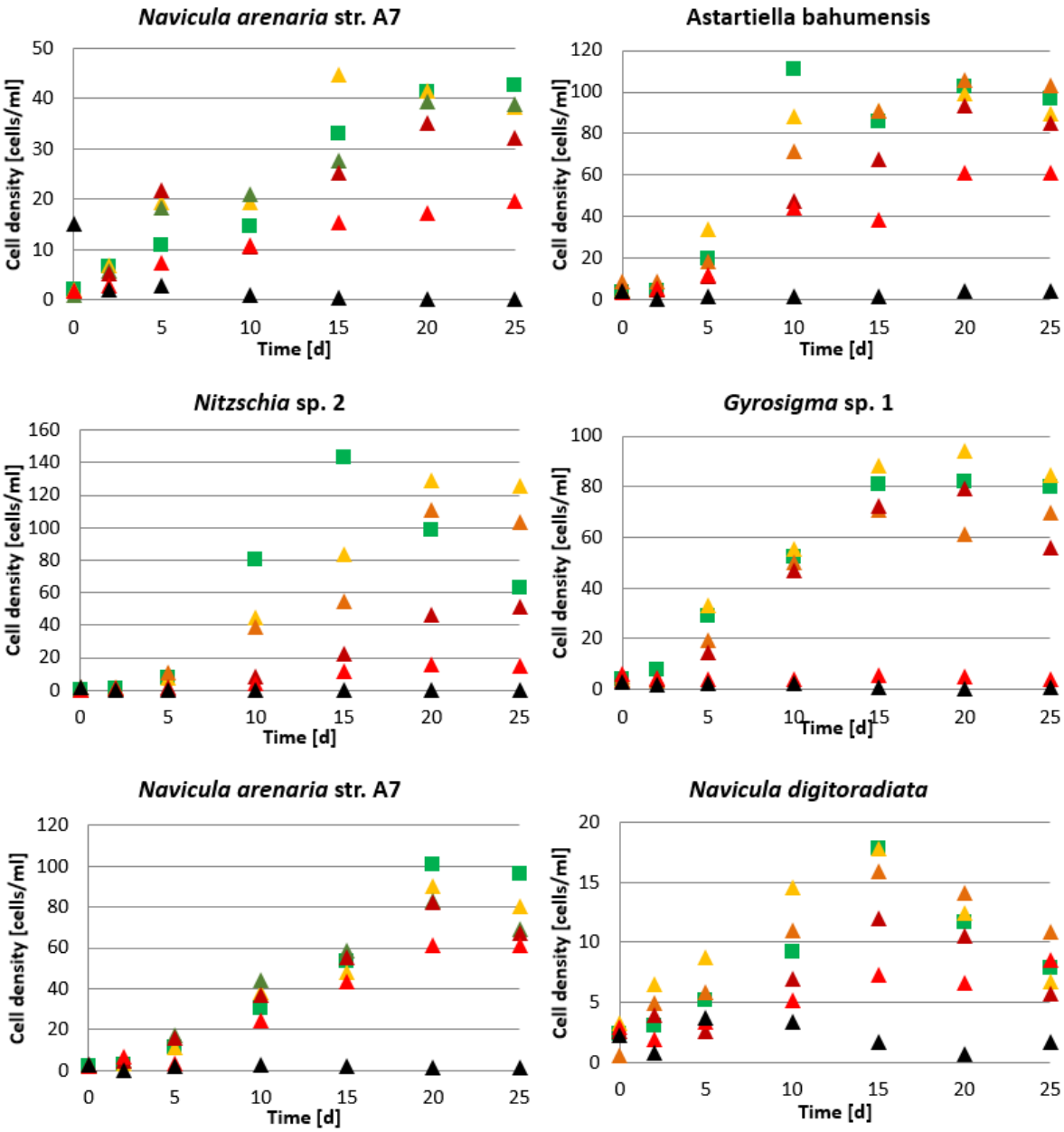


Fig. S10 a: Cell densities per species in the atrazine experiment. Colour codes refer the atrazine concentrations (green = control. yellow = 50 µg/L. orange = 100 µg/L. dark red = 200 µg/L. red = 500 µg/L. black = 1000 µg/L).


Cylindrotheca fusiformis
Nitzschia sp.1
Nitzschia sp. 3
Amphora lineolata
Cylindrotheca closterium
Gyrosigma sp. 2
Cell density [cells/ml]
Time [d]

3988


Navicula arenaria str. A7
Cell density [cells/ml]
Time [d]
Astartiella bahumensis
Cell density [cells/ml]
Time [d]
Nitzschia sp. 2
Cell density [cells/ml]
Time [d]
Gyrosigma sp. 1
Cell density [cells/ml]
Time [d]
Navicula arenaria str. A7
Cell density [cells/ml]
Time [d]
Navicula digitoradiata
Cell density [cells/ml]
Time [d]

3989

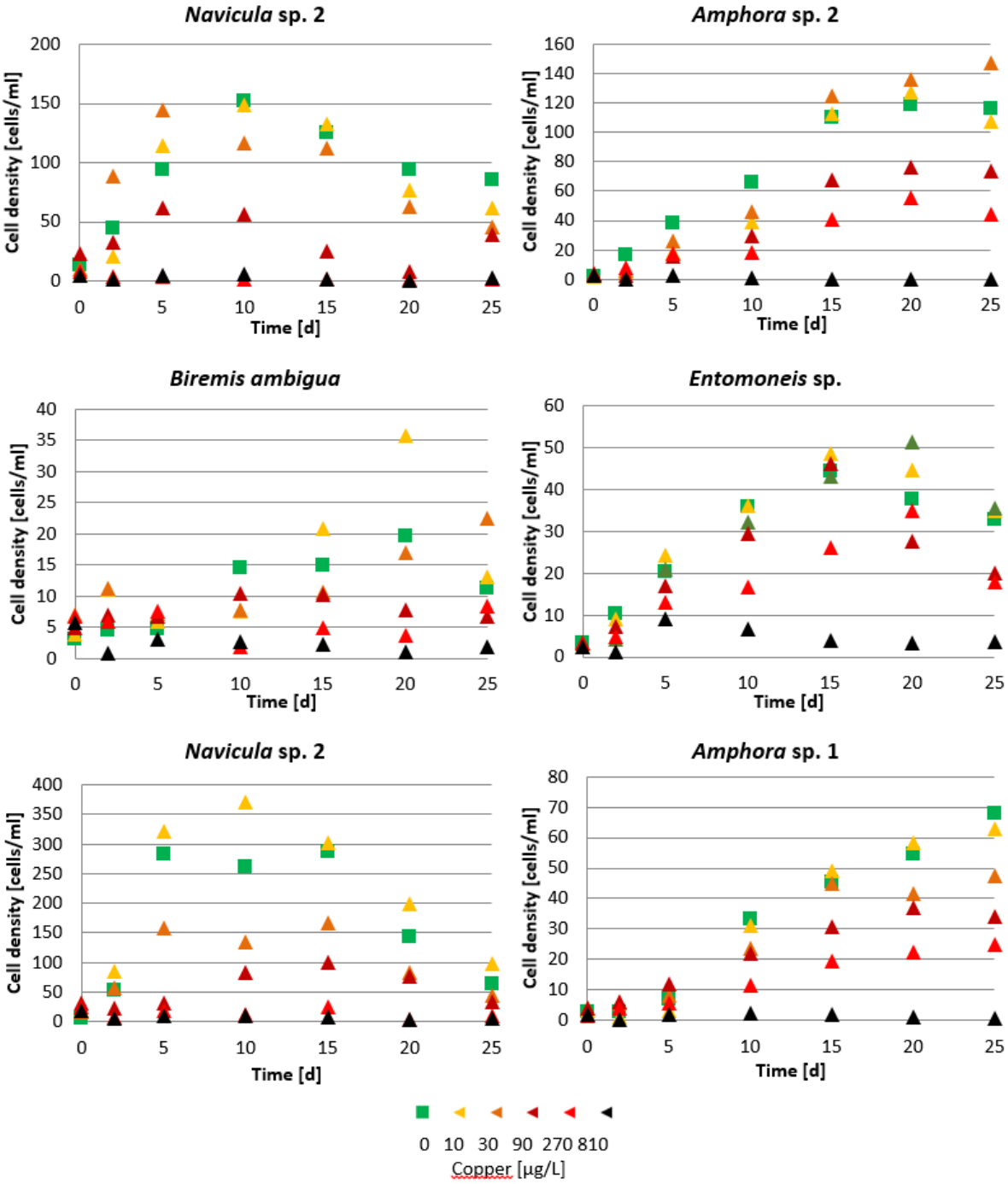


Fig. S10 b: Cell densities per species in the copper experiment. Colour codes refer the copper concentrations (green = control. yellow = 10 µg/L. orange = 30 µg/L. dark red = 90 µg/L. red = 270 µg/L. black = 810 µg/L).

Addendum III – Supporting Material Chapter 4

Community	Species	Acc. Nr.	Biovolume total [10^3 mm^3]	Biovolume / cell [μm^3]	EPS total [mg/ml]	EPS / biomass [mg/mm^3]
A	*Nitzschia* sp. 1	DCG 0483	31.46 (+- 3.09)	380 (+- 48)	0.25 (+- 0.01)	7.90 (+- 0.49)
	Nitzschia acicularis	DCG 0459	25.39 (+- 3.08)	517 (+- 68)	0.31 (+- 0.01)	12.43 (+- 1.76)
	Amphora lineolata	DCG 0478	18.47 (+- 2.82)	867 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)
	Cylindrotheca closterium		35.68 (+- 2.81)	345 (+- 60)	0.32 (+- 0.03)	9.16 (+- 1.68)
	Gyrosigma sp. 2	DCG 0491	43.52 (13.67)	2081 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)
	Navicula arenaria	DCG 0487	78.88 (18.50)	5644 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)
B	*Gyrosigma* sp. 1	DCG 0468	20.62 (+- 5.49)	2147 (+- 509)	1.56 (+- 0.42)	75.88 (+- 5.15)
	Entomoneis sp.	DCG 0466	87.72 (+- 9.33)	10932 (+- 2743)	0.16 (+- 0.02)	1.93 (+- 0.30)
	Navicula arenaria	DCG 0487	78.88 (18.50)	5644 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)
	Amphora lineolata	DCG 0478	18.47 (+- 2.82)	867 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)
	Gyrosigma sp. 2	DCG 0491	43.52 (13.67)	2081 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)
	Navicula digitoradiata	DCG 0490	36.32 (+- 6.86)	5738 (+- 528)	1.36 (+- 0.31)	39.19 (+- 14.76)

Table S1: Listing of the two diatom communities used in the experiments. From left to right are indicted: species name, strain accession number ('Acc. Nr. ') in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be), biovolume (total and per cell) and EPS production (total and per biomass) in monoculture (all n=3).

Substance	Theoretical concentration [µg/l]	Measured Concentration [µg/l]
Control	<1	<2.6
Atrazine	200	160
Atrazine	500	542
Copper	200	271
Copper	500	508

Table S2: Experimental atrazine and copper treatments with indication of theoretical and verified atrazine and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of quantification : < 1,0 µg/L atrazine and 2.6 µg/L copper for 10 ml samples).

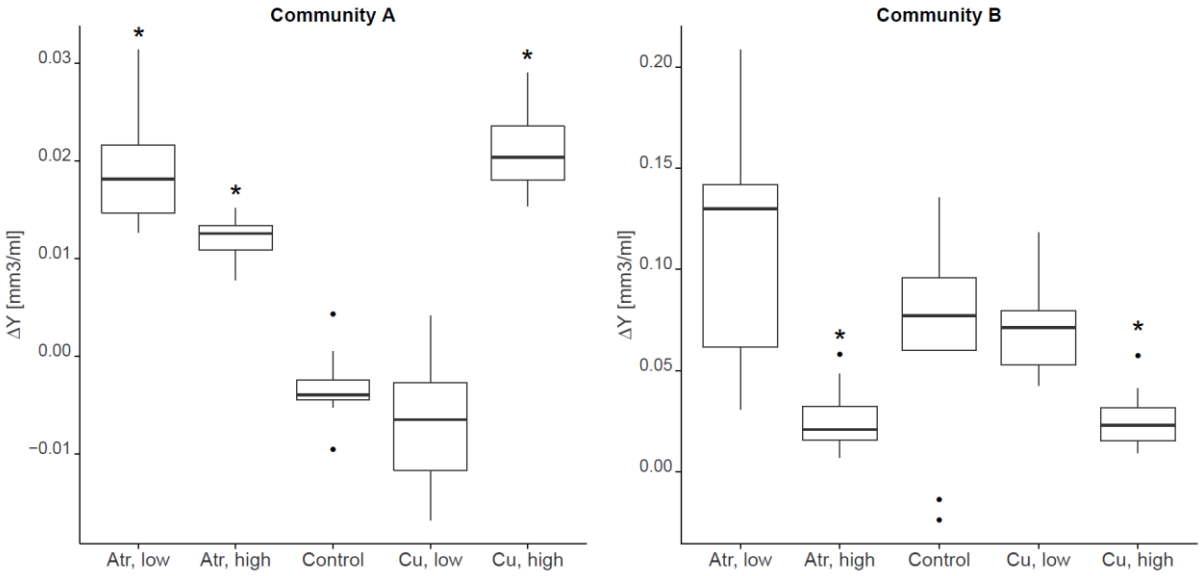


Fig. S1: The net biodiversity effect in two benthic diatom communities (A and B) exposed to low and high (200 and 500 µg/L) concentrations of copper and atrazine. ΔY is the difference between the biomass yield observed in diatom communities and the yield expected from the individual species' monocultures. Asterisks (*) denote significant differences in the net biodiversity effect compared to the respective control.

Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr, low	0.0229	0.0024	9.60	**<0.0001**	1	-286	149	Yes	8.59	0.0723
	Atr, high	0.0156	0.0024	6.36	**<0.0001**						
	Cu, low	0.0039	0.0024	-1.64	0.1096						
	Cu, high	0.0242	0.0024	10.17	**<0.0001**						
	Atr, low	0.0229	0.0025	9.11	**<0.0001**	2	-287	154	Yes		
	Atr, high	0.0152	0.0015	9.84	**<0.0001**						
	Cu, low	-0.0039	0.0025	-1.53	0.1327						
	Cu, high	0.0242	0.0020	12.13	**<0.0001**						
B	Atr, low	0.0044	0.0189	2.33	**0.0252**	1	-121	66.4	No	25.2	<0.0001
	Atr, high	-0.0396	0.0189	-2.10	**0.0425**						
	Cu, low	0.0043	0.0189	0.22	0.8228						
	Cu, high	-0.0398	0.0189	-2.11	**0.0415**						
	Atr, low	0.0439	0.0277	1.58	0.1215	2	-138	79.1	Yes		
	Atr, high	-0.0396	0.0188	-2.11	**0.0414**						
	Cu, low	0.0043	0.0195	-0.22	0.8284						
	Cu, high	-0.0398	0.0186	-2.14	**0.0384**						

Table S3: Results of generalized least squared models predicting changes in the net biodiversity effect under copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the net biodiversity effect and treatment type, i.e. the difference of the net biodiversity effect (in mm^3/ml) at each stress level compared to the control. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote

the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr, low	0.0038	0.0005	6.96	**<0.0001**	1	-405	208	Yes	7.23	0.1244
	Atr, high	0.0112	0.0005	20.58	**<0.0001**						
	Cu, low	-0.0099	0.0005	-18.21	**<0.0001**						
	Cu, high	0.0094	0.0005	17.31	**<0.0001**						
	Atr, low	0.0038	0.0005	8.05	**<0.0001**	2	-404	211	Yes		
	Atr, high	0.0112	0.0005	23.08	**<0.0001**						
	Cu, low	-0.0099	0.0006	-15.94	**<0.0001**						
	Cu, high	0.0094	0.0004	26.00	**<0.0001**						
B	Atr, low	0.0050	0.0016	3.11	**0.0035**	1	-319	165	Yes	15.28	0.0042
	Atr, high	0.0021	0.0016	-1.32	0.1939						
	Cu, low	0.0013	0.0016	0.85	0.4030						
	Cu, high	0.0023	0.0016	1.44	0.1575						
	Atr, low	0.0049	0.0022	2.26	**0.0292**	2	-326	173	Yes		
	Atr, high	0.0021	0.0014	1.43	0.1597						
	Cu, low	0.0013	0.0017	0.80	0.4259						
	Cu, high	0.0023	0.0015	1.55	0.1301						

Table S4: Results of generalized least squared models predicting changes in the dominance effect under copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the dominance effect and treatment type, i.e. the difference of the dominance effect (in mm^3/ml) at each stress level compared to the control. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr, low	0.0191	0.0022	8.67	**<0.0001**	1	-293	152	Yes	9.31	0.0537
	Atr, high	0.0040	0.0022	1.80	0.0799						
	Cu, low	0.0060	0.0022	2.73	**0.0094**						
	Cu, high	0.0148	0.0022	6.73	**<0.0001**						
	Atr, low	0.0191	0.0024	7.83	**<0.0001**	2	-294	157	Yes		
	Atr, high	0.0040	0.0014	2.92	**0.0057**						
	Cu, low	0.0060	0.0021	2.88	**0.0064**						
	Cu, high	0.0148	0.0019	7.64	**<0.0001**						
B	Atr, low	0.0390	0.0178	2.19	**0.0300**	1	-125	69	No	21.91	0.0002
	Atr, high	-0.0417	0.0178	-2.34	**0.0242**						
	Cu, low	0.0029	0.0178	0.16	0.8707						
	Cu, high	-0.0421	0.0178	-2.37	**0.0229**						
	Atr, low	0.0390	0.0258	1.51	0.1392	2	-139	79	Yes		
	Atr, high	-0.0417	0.0177	-2.36	**0.0234**						
	Cu, low	0.0029	0.0184	0.16	0.8749						
	Cu, high	-0.0421	0.0175	-2.40	**0.0210**						

Table S5: Results of generalized least squared models predicting changes in the complementarity effect under copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the complementarity effect and treatment type, i.e. the difference of the complementarity effect (in mm^3/ml) at each stress level compared to the control. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Com	Effect	Stress	Slope	s.e.	T	**P**	Mod	AIC	Log lik.	Validity	LR	P LR
A	TIC	Atr, low	0.0189	0.0026	7.08	**<0.0001**	1	-278	145	Yes	3.99	0.4076
		Atr, high	0.0090	0.0026	3.27	**0.0022**						
		Cu, low	0.0072	0.0026	2.72	**0.0096**						
		Cu, high	0.0141	0.0026	5.32	**<0.0001**						
		Atr, low	0.0189	0.0027	6.99	**<0.0001**	2	-274	147	Yes		
		Atr, high	0.0090	0.0021	4.18	**0.0002**						
		Cu, low	0.0072	0.0021	3.44	**0.0014**						
		Cu, high	0.0141	0.0025	5.68	**<0.0001**						
	TDC	Atr, low	0.0004	0.0011	0.34	0.7318	1	-349	180	No	32.73	<0.0001
		Atr, high	-0.0047	0.0011	-4.21	**0.0001**						
		Cu, low	-0.0012	0.0011	-1.06	0.2964						
		Cu, high	0.0008	0.0011	0.68	0.5012						
		Atr, low	0.0004	0.0006	0.69	0.4907	2	-372	196	Yes		
		Atr, high	-0.0047	0.0013	-3.55	**0.0010**						
		Cu, low	-0.0012	0.0004	-2.78	**0.0082**						
		Cu, high	0.0008	0.0010	0.78	0.4402						
B	TIC	Atr, low	0.0391	0.0292	1.34	0.1885	1	-86	49	Yes	19.16	0.0007
		Atr, high	-0.018	0.0292	-0.60	0.5529						
		Cu, low	0.1567	0.0292	5.36	**<0.0001**						
		Cu, high	0.0311	0.0292	1.06	0.2938						
		Atr, low	0.0391	0.0184	2.12	**0.0400**	2	-97	59	Yes		
		Atr, high	-0.018	0.0151	-1.16	0.2539						
		Cu, low	0.1567	0.0337	4.64	**<0.0001**						
		Cu, high	0.0311	0.0306	1.02	0.3150						
	TDC	Atr, low	-0.0002	0.0233	-0.01	0.9940	1	-104	58	No	51.18	<0.0001
		Atr, high	-0.0242	0.0233	-1.04	0.3063						
		Cu, low	-0.1538	0.0233	-6.60	**<0.0001**						
		Cu, high	-0.0732	0.0233	-3.14	**0.0032**						
		Atr, low	-0.0002	0.0087	-0.02	0.9837	2	-147	84	Yes		
		Atr, high	-0.0242	0.0047	-5.18	**<0.0001**						
		Cu, low	-0.1538	0.0279	-5.52	**<0.0001**						
		Cu, high	-0.0732	0.0230	-3.18	**0.0028**						

Table S6: Results of generalized least squared models predicting changes in trait-independent complementarity (TIC) and trait-dependent complementarity (TDC) under copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Effect' indicates the complementarity effect (TIC or TDC). 'Stress' denotes which type of stress diatoms were exposed to: high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the respective complementarity effect and treatment type, i.e. the difference of the complementarity effect

(in mm^3/ml) at each stress level compared to the control. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted (('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr	1.2e-3	3.7e-4	3.16	**0.0158**	1	-44	25	Yes	0.43	0.81
		9.1e-4	3.3e-4	2.76	**0.0282**	2	-40	25	Yes		
	Cu	-2.6e-4	4.2e-4	-0.64	0.5452	1	-36	21	Yes	3.18	0.20
		1.1e-4	1.9e-4	0.55	0.5964	2	-37	23	Yes		
B	Atr	1.4e-3	5.1e-3	0.28	0.7884	1	-14	10	Yes	5.04	0.08
		-6.5e-3	2.2e-3	3.03	**0.0192**	2	-15	12	Yes		
	Cu	1.2e-2	3.6e-3	3.96	**0.0115**	1	-5.0	5.5	Yes	3.66	0.16
		2.1e-2	2.7e-3	7.59	**0.0001**	2	-6.0	8.0	Yes		

Table S7: Results of generalized least squared models predicting changes in trait-independent complementarity (TIC) from diatom EPS production. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: copper (Cu) and atrazine (Atr). Models were fitted over the three concentrations of each stressor (control, 200 and 500 µg/L). 'Slope' indicates the relation between EPS production and TIC. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Com	Stress	Pred	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr	Tol	-0.27	0.21	-1.30	0.2240	1	20.0	-7.0	Yes	<0.01	0.95
			-0.27	0.21	-1.30	0.2213	2	22.0	-7.0	Yes		
		Yield	-0.23	0.16	-1.38	0.1983	1	20.3	-7.2	No	0.01	0.92
			-0.23	0.16	-1.39	0.1937	2	-22.3	-7.1	No		
	Cu	Tol	-0.19	0.25	-0.78	0.4557	1	29.2	-11.6	Yes	0.40	0.53
			-0.21	0.26	-0.79	0.4501	2	30.9	-11.4	Yes		
		Yield	-0.25	0.23	-1.10	0.2982	1	28.8	-11.4	Yes	0.44	0.51
			-0.27	0.24	-1.13	0.2841	2	30.4	-11.2	Yes		
B	Atr	Tol	-0.41	0.31	-1.33	0.2107	1	21.2	-7.6	Yes	0.19	0.66
			-0.44	0.30	-1.47	0.1735	2	23.0	-7.5	Yes		
		Yield	-0.21	0.23	-0.89	0.3960	1	22.8	-8.4	Yes	0.11	0.75
			-0.22	0.23	-0.97	0.3540	2	24.7	-8.3	Yes		
	Cu	Tol	-0.78	0.21	-3.80	**0.0035**	1	19.3	-6.9	Yes	0.21	0.65
			-0.80	0.20	-3.99	**0.0026**	2	21.2	-6.6	Yes		
		Yield	-0.54	0.18	-2.99	**0.0136**	1	22.4	-8.2	Yes	0.08	0.77
			-0.55	0.18	-3.07	**0.0118**	2	24.3	-8.2	Yes		

Table S8: Results of generalized least squared models predicting the deviation of the observed frequency from the observed relative yield of diatoms grown in atrazine- and copper-exposed communities from the species' monoculture yields and stress tolerance . 'Com' denotes for which diatom community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: copper (Cu) and atrazine (Atr). Models were fitted over the two concentrations of each stressor (200 and 500 µg/L). 'Pred' denotes the predictor: species monoculture yield ('Yield') or stress tolerance ('Tol'). 'Slope' indicates the relation between the deviation of the species' observed frequency from their observed relative yield and their monoculture yield and stress tolerance. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

Addendum IV – Supporting Material Chapter 5

Species	Acc. Nr.	Biovolume total [10^3 mm^3]	Biovolume / cell [μm^3]	EPS total [mg/ml]	EPS / biomass [mg/mm^3]	EFA total [µg/ml]	EFA / biomass [µg / mm^3]
***Nitzschia* sp.**	DCG 0483	31.46 (+- 3.09)	380.34 (+- 48)	0.25 (+- 0.01)	7.90 (+- 0.49)	5.80 (+- 0.58)	184.47 (+- 4.31)
Nitzschia acicularis	DCG 0459	25.39 (+- 3.08)	517.58 (+- 68)	0.31 (+- 0.01)	12.43 (+- 1.76)	2.53 (+- 0.63)	98.67 (+- 13.62)
Amphora lineolata	DCG 0478	18.47 (+- 2.82)	867.75 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)	0.33 (+- 0.08)	18.34 (+- 7.59)
Cylindrotheca closterium		35.68 (+- 2.81)	345.20 (+- 60)	0.32 (+- 0.03)	9.16 (+- 1.68)	0.46 (+- 0.17)	13.17 (+- 5.24)
***Gyrosigma* sp.**	DCG 0491	43.52 (+-13.67)	2081.56 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)	0.70 (+- 0.21)	16.23 (+- 3.44)
Navicula arenaria	DCG 0487	78.88 (+-18.50)	5644.09 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)	3.29 (+- 0.81)	42.13 (+- 8.27)

Table S1: Listing of the six diatom strains used in the experiments. From left to right are indicated: species name, strain accession number ('Acc. Nr. ') in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be), biovolume (total and per cell), EPS and EFA production (total and per biovolume) in monoculture (all n=3).

Substance	Theoretical concentration [µg/l]	Measured Concentration [µg/l]
Control	<1	<2.6
Atrazine	200	160
Atrazine	500	542
Copper	200	271
Copper	500	508

Table S2: Experimental atrazine and copper treatments with indication of theoretical and verified atrazine and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of quantification : < 1,0 µg/L atrazine and 2.6 µg/L copper for 10 ml samples).

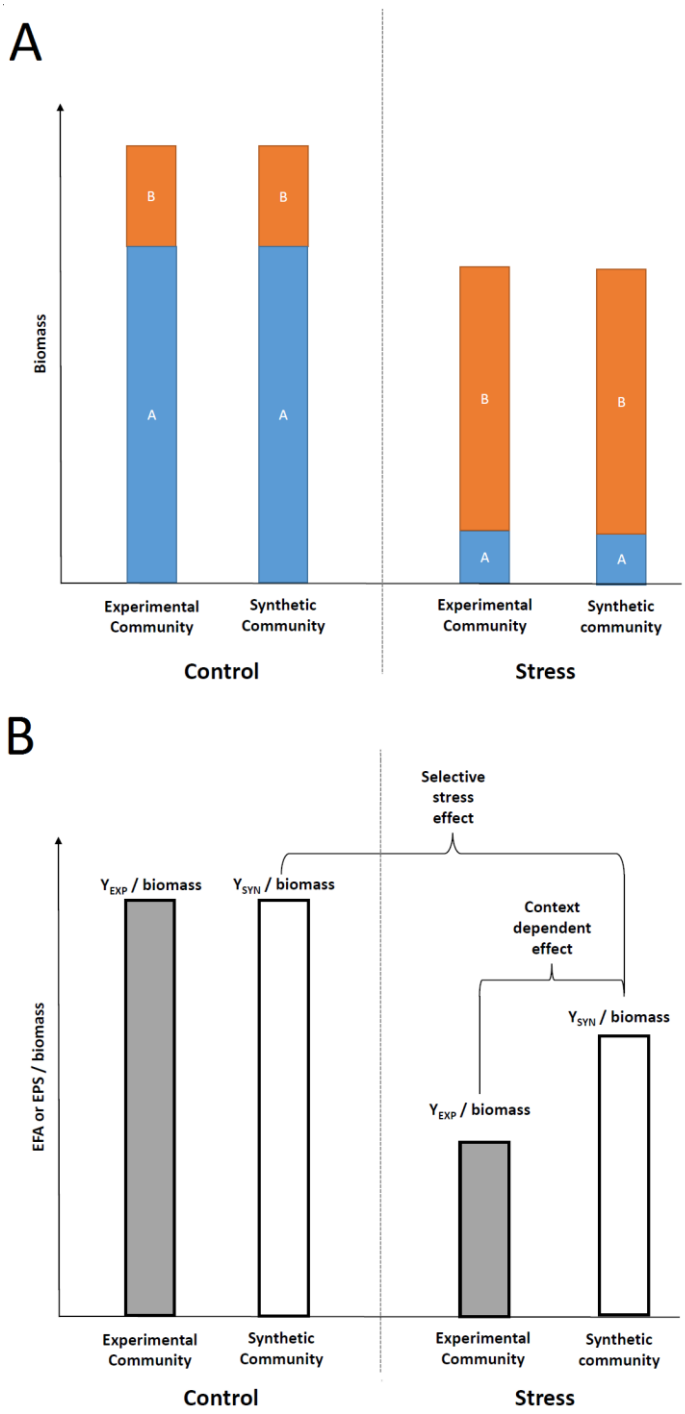


Fig. S1: Quantification of selective and context-dependent stress effects by comparison of the EFA and EPS production in experimental and synthetic communities. For the sake of clarity, this example uses a community with only two species (A and B) and only one stress level. Panel A shows the community structure of experimental and synthetic communities. In the experimental community, stress induces a change in biomass and community structure (dominance of species B under stress). The synthetic community reflects the biomass and community structure observed in the corresponding experimental community. Panel B shows the EFA or EPS production per unit biomass in the experimental community (Y_{EXP}) and in the synthetic community (Y_{SYN}). Y_{SYN} is calculated by multiplying each species' EFA or EPS production in unstressed monocultures with its biomass share in the corresponding experimental community. Y_{SYN} thus reflects the EFA or EPS production expected in a community without stress exposure and species interactions, but which has the same biomass and community structure as induced by stress. Note that for the sake of simplicity, Y_{EXP} and Y_{SYN} in the control are shown with the same value, but this does

not necessarily have to be the case. Selective stress effects are quantified by comparing synthetic communities reflecting the community structure under stress and under control conditions. Confounding effects of direct stress or changes in species interactions are eliminated, since Y_{SYN} of both communities is calculated from unstressed monocultures. Context-dependent effects are quantified by comparing experimental and synthetic communities of the same community structure. Confounding effects of community structure are eliminated, and differences in Y_{EXP} and Y_{SYN} are attributed to species interactions or direct stress effects on Y_{EXP}. In this example, Y_{EXP} is lower under stress than in the control. This is due to both context-dependent stress effects (Y_{EXP} stress < Y_{SYN} stress) and selective stress effects (Y_{SYN} stress < Y_{SYN} control). Here, stress would thus reduce diatom EFA or EPS production by reducing the species' EFA or EPS output, as well as by causing dominance by an unproductive species.

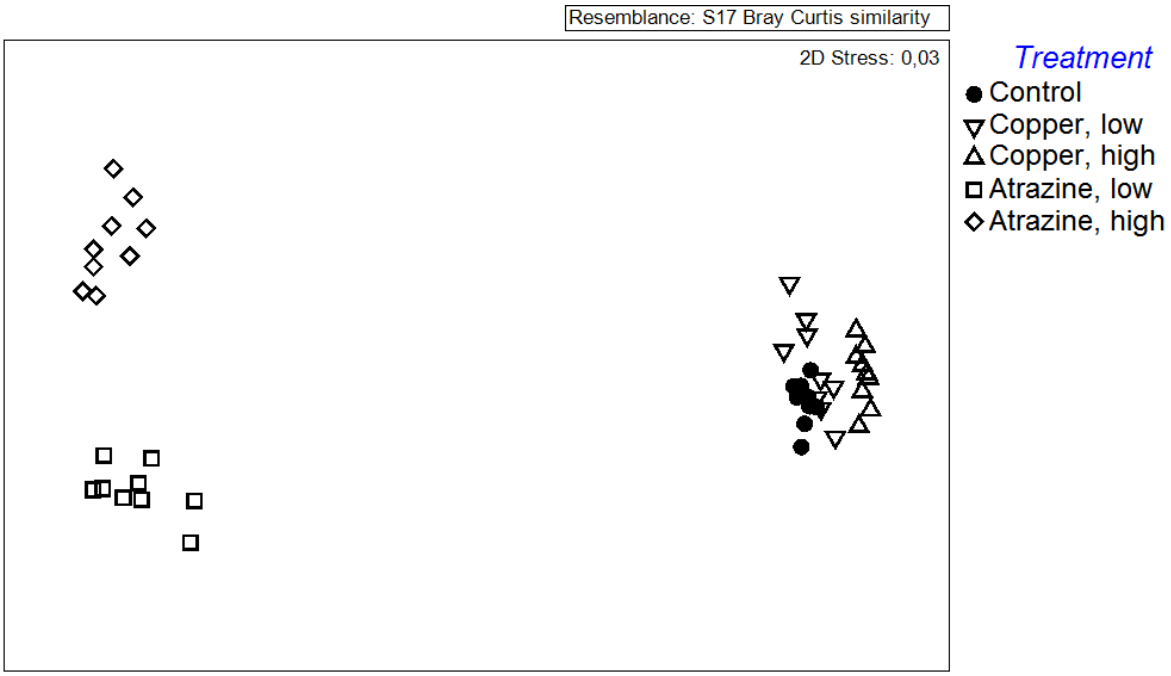


Fig. S2: Non-metric multidimensional scaling (Bray-Curtis similarity) of untransformed relative (%) biomass data of communities from unstressed conditions and exposed to low (200 µg/L) and high (500 µg/L) atrazine and copper concentrations.

Treatments	Dissimilarity	Species	Contribution
Copper, low	14.49	*Navicula arenaria*	33.25
		Nitzschia acicularis	33.00
		Gyrosigma sp.	10.92
Copper, high	18.30	*Navicula arenaria*	28.56
		Nitzschia sp.	19.60
		Nitzschia acicularis	16.51
Atrazine, low	69.79	*Cylindrotheca closterium*	49.63
		Nitzschia acicularis	28.45
		Navicula arenaria	12.71
Atrazine, high	75.97	*Nitzschia acicularis*	39.20
		Cylindrotheca closterium	25.23
		Navicula arenaria	24.79

Table S3. Results of dissimilarity percentage (SIMPER) analysis of biomass composition of diatom communities grown exposed to copper and atrazine compared to control communities. Analysis was run on the untransformed biomass data. 'Treatments' indicates the treatment whose biomass composition is compared to the control: low (200 µg/L) and high (500 µg/L) atrazine and copper. 'Dissimilarity' indicates the average dissimilarity (in %) of biomass composition of the respective treatment and the control. 'Species' indicates the three species contributing most to dissimilarities with the control biomass composition. 'Contribution' indicates the average contribution (in %) of each species to dissimilarity from the control biomass composition.

Treatment	Similarity	Species	Mean	Contribution
Control	91.52	*Nitzschia acicularis*	20.01	48.02
		Navicula arenaria	15.17	32.56
		Nitzschia sp.	2.78	5.93
Copper, low	84.07	*Nitzschia acicularis*	18.30	49.25
		Navicula arenaria	12.69	33.26
		Nitzschia sp.	2.70	7.47
Copper, high	90.06	*Nitzschia acicularis*	20.96	62.52
		Navicula arenaria	11.62	33.10
Atrazine, low	88.32	*Cylindrotheca closterium*	36.15	76.27
		Navicula arenaria	7.44	9.85
		Gyrosigma sp.	4.78	3.78
Atrazine, high	86.27	*Cylindrotheca closterium*	15.68	74.22
		Gyrosigma sp.	2.76	11.90
		Navicula arenaria	2.46	9.06

Table S4: Results of similarity percentage (SIMPER) analysis of biomass composition of diatom communities grown under unstressed conditions as well as copper and atrazine exposure. Analysis was run on the untransformed biomass data. 'Treatment' indicates the growth conditions: control, low (200 µg/L) and high (500 µg/L) atrazine and copper. 'Similarity' indicates the average similarity (in %) of biomass composition within the nine replicates of each treatment. 'Species' indicates the species contributing most to similarities within treatments (only species with a mean biomass > 2 x 10^3 µm^3 are reported). 'Mean' indicates the mean biomass (in 10^3 µm^3) of species within treatments. 'Contribution' indicates the contribution (in %) of each species to similarities within treatments.

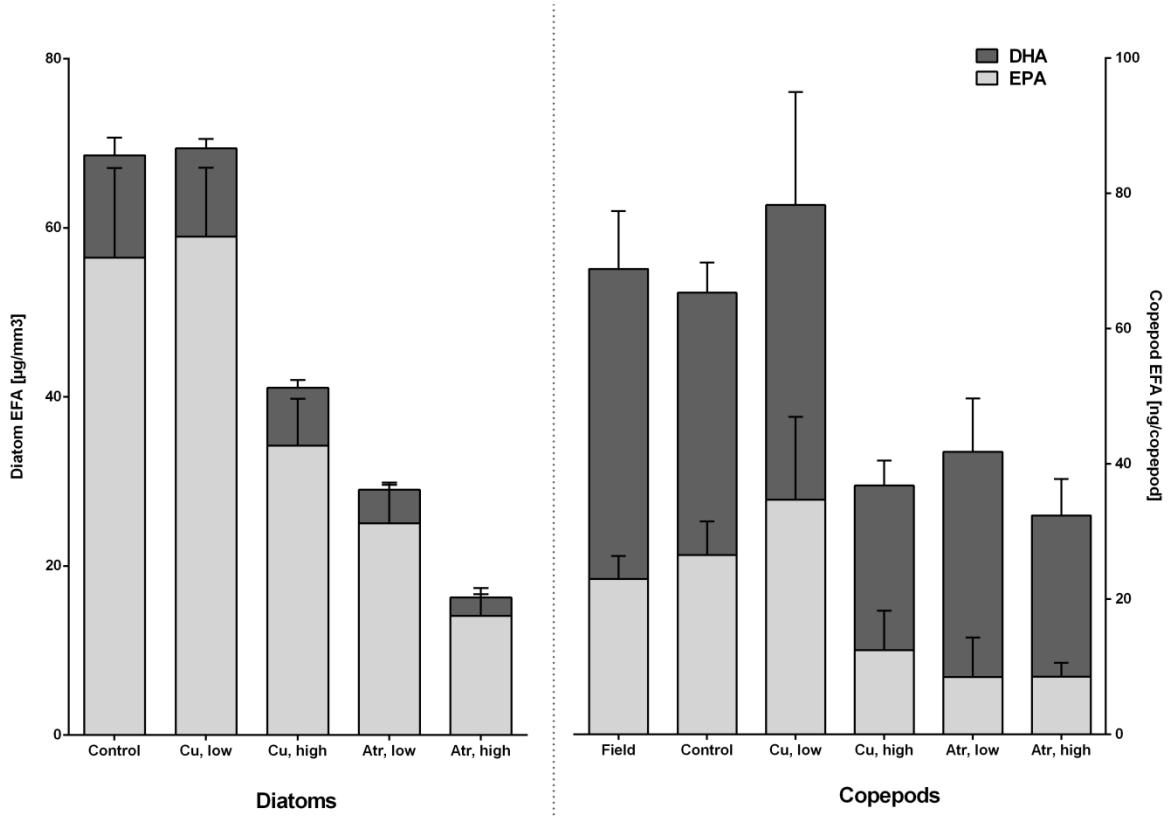


Fig. S3: EPA and DHA content of diatom communities exposed to low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') concentrations, and EPA and DHA content of *Microarthridion littorale* collected in the field and after 10d of feeding on the diatom diet that was subject to the stressors (see left part of the figure for corresponding abbreviations of the treatments).

Addendum V – Acute toxicity of copper to harpacticoid copepods

Addendum V reports the results of single-species toxicity tests, which measured the tolerance of five harpacticoid copepod species to direct copper exposure.

Harpacticoid copepods were collected from sediments at the Paulina intertidal flat (SW Netherlands, 51° 21'N, 3°43'E). Five harpacticoid copepod species (*Microathridion littorale*, *Stenhelia* sp., *Platychelipus littoralis*, *Nannopus palustris* and one species of the family Ectinosomatidae) were extracted alive from the sediment using a mixed technique of sediment decantation and extraction via white light attraction. Adult specimens were randomly collected with a glass pasteur pipette using a Wild M5 binocular. Copepods were washed 3 times over a 38 µm sieve and placed in glass jars with filtered and autoclaved natural seawater overnight in order to empty their intestines prior to the start of the experiment.

Copepod sensitivity to direct copper exposure was quantified by exposing the five harpacticoid species collected at the sampling site for 48h to a concentration gradient of 0 to 2430 µg/L copper (Table S1). The experiment was conducted in glass jars containing 100 ml of filtered and autoclaved seawater in a climate room at 15±1 °C with a 12:12 h light:dark cycle and 40 to 50 µmol photons m^{-2} s^{-1}. During the experiment, harpacticoids were offered 0.5 mm^3 of an equal mix of the six diatom species used as food source in Chapter 5 (*Nitzschia* sp., *N. acicularis, C. closterium*, *A. lineolata, Gyrosigma* sp.*, N. arenaria*).

Cu [µg/l]	Replicates (copepod individuals per replicate)				
	Microarthridion littorale	*Stenhelia* sp.	*Nannopus palustris*	Ectinosomatidae sp.	*Platychelipus littoralis*
0	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
10	5 (15)				5 (30)
30	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
90	5 (15)				5 (30)
270	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
810	5 (15)	5 (15)	5 (15)		5 (30)
2430	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)

Table S1: Experimental copper treatments with indication of the number of replicates per treatment and the number of individuals per replicate (in brackets) for each copepod species (*Microarthridion littorale*, *Stenhelia* sp., *Nannopus palustris*, Ectinosomatidae sp., *Platychelipus littoralis*)

Copepod sensitivity to direct copper exposure was quantified as the 48-h median lethal concentration (LC50), i.e. the effective copper concentration inducing a 50% mortality of each

harpacticoid species. LC50 values were calculated by means of a three-parameter log-logistic model (Equation 1) and a three-parameter Weibull model in two different parameterisations (Equations 2 and 3) as described in Ritz and Streibig (2005).

$$Y = d / (1 + \exp\{b[\log x - \log e]\}) \quad [1]$$

$$Y = d(1 - \exp\{ - \exp[b(\log x - \log e)]\}) \quad [2]$$

$$Y = d(\exp\{ - \exp[b(\log x - e)]\}) \quad [3]$$

Y represents the response variable (surviving harpacticoid individuals), *b* the relative slope of the curve, *d* the upper limit, *e* the inflection point, and *x* the copper concentration. Dose-response models and LC50 values were computed in R 3.0.1. using RStudio (R Development Core Team 2016) and the package drc (Ritz and Streibig 2005).

The five harpacticoid copepod species differed in their sensitivity to direct copper exposure, with LC50 values ranging from 387 µg/L (*N. palustris*) to 1475 µg/L (*Stenhelia* sp.). *M. littorale*, being the most abundant copepod species at the sampling site representing ~ 90% of all collected individuals, was the second most copper-sensitive species with an LC50 of 546 µg/L (Table S2).

Species	LC50	Surviving individuals per treatment (µg/L Cu)						
		Control	10	30	90	270	810	2430
Microarthridion littorale	546.43 (+-78.38)	65	67	68	66	48	18	14
Stenhelia sp..	1474.60 (+-235.58)	71		70		57	55	14
Nannopus palustris	386.83 (+-160.89)	67		67		40	23	0
Ectinosomatidae sp.	696.88 (+-188.42)	66		50		48	33	2
Platychelipus littoralis	879.28 (+-260.09)	134	133	107	116	115	67	12

Table S2: Copper 48h-LC50 values obtained for five harpacticoid copepod species (standard deviations are indicated in brackets). Each treatment had five replicates, with 15 individuals (30 for *Platychelipus littoralis*) harpacticoid individuals per replicate. Surviving individuals are indicated as sum of the five replicates.

References

Aarnio, K., and E. Bonsdorff. 1993. Seasonal variation in abundance and diet of the sand goby *Pomatoschistus minutus* (Pallas) in a northern Baltic archipelago. Ophelia 37:19–30.

Abdulkadir, S., and M. Tsuchiya. 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. Journal of Experimental Marine Biology and Ecology 354:1–8.

Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone, and S. Lavorel. 2010. Intraspecific functional variability: Extent, structure and sources of variation. Journal of Ecology 98:604–613.

Alheit, J., and W. Scheibel. 1982. Benthic harpacticoids as a food source for fish. Marine Biology 70:141–147.

Alyahya, H., A. H. El-Gendy, S. Al Farraj, and M. El-Hedeny. 2011. Evaluation of heavy metal pollution in the Arabian Gulf using the clam *Meretrix meretrix* Linnaeus, 1758. Water, Air, and Soil Pollution 214:499–507.

Amin, S. A., M. S. Parker, and E. V. Armbrust. 2012. Interactions between diatoms and bacteria. Microbiology and Molecular Biology Reviews 76:667–684.

Andersen, B. S., J. D. Carl, P. Grønkjær, and J. G. Støttrup. 2005. Feeding ecology and growth of age 0 year *Platichthys flesus* (L.) in a vegetated and a bare sand habitat in a nutrient rich fjord. Journal of Fish Biology 66:531–552.

Anderson, P. J., and J. F. Piatt. 1999. Community reorganisation in the Gulf of Alaska following ocean climate regime shift. Marine Ecology Progress Series 189:117–123.

Arendt, K. E., S. H. Jónasdóttir, P. J. Hansen, and S. Gärtner. 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. Marine Biology 146:513–530.

Arndt, C., and U. Sommer. 2014. Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a discussion about their suitability for marine fish larvae. Aquaculture Nutrition 20:44–59.

Arts, M. T., and B. C. Wainmann. 1999. Lipids in Freshwater Ecosystems. Springer, New York.

Azam, F., and A. Z. Worden. 2004. Microbes, molecules and marine ecosystems. Science 303:1622-1624.

Backhaus, T., J. Snape, and J. Lazorchak. 2012. The impact of chemical pollution on biodiversity and ecosystem services: The need for an improved understanding. Integrated Environmental Assessment and Management 8:575–576.

Baert, J. M., C. R. Janssen, K. Sabbe, and F. De Laender. 2016. Per capita interactions and stress tolerance drive stress-induced changes in biodiversity effects on ecosystem functions. Nature Communications 7.

Bai, J., R. Xiao, B. Cui, K. Zhang, Q. Wang, X. Liu, H. Gao, and L. Huang. 2011. Assessment of heavy metal pollution in wetland soils from the young and old reclaimed regions in the Pearl River Estuary, South China. Environmental Pollution 159:817–824.

Baird, D. J., and P. J. Van den Brink. 2007. Using biological traits to predict species sensitivity to toxic substances. Ecotoxicology and Environmental Safety 67:296–301.

Ball, B. A., M. D. Hunter, J. S. Kominoski, C. M. Swan, and M. A. Bradford. 2008. Consequences of non-random species loss for decomposition dynamics: Experimental evidence for additive and non-additive effects. Journal of Ecology 96:303–313.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9:1146–56.

Barnett, A., V. Méléder, L. Blommaert, B. Lepetit, P. Gaudin, W. Vyverman, K. Sabbe, C. Dupuy, and J. Lavaud. 2015. Growth form defines physiological photoprotective capacity in intertidal benthic diatoms. International Society for Microbial Ecology 9:1–14.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth mass extinction already arrived? Nature 471:51–57.

Barranguet, C., and J. Kromkamp. 2000. Estimating primary production rates from photosynthetic electron transport in estuarine microphytobenthos. Marine Ecology Progress Series 204:39–52.

Bartell, S. M. 2000. Are ecosystems hormetic? Human and Ecological Risk Assessment 6:237–243.

Becker, J., N. Eisenhauer, S. Scheu, and A. Jousset. 2012. Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. Ecology Letters 15:468–474.

Bejarano, A. C., and G. T. Chandler. 2003. Reproductive and developmental effects of atrazine on the estuarine meiobenthic copepod *Amphiascus tenuiremis*. Environmental Toxicology and

Chemistry / SETAC 22:3009–16.

Bejarano, A. C., P. L. Pennington, M. E. Delorenzo, and G. T. Chandler. 2005. Atrazine effects on meiobenthic assemblages of a modular estuarine mesocosm. Marine Pollution Bulletin 50:1398–1404.

Beketov, M. A., K. Foit, R. B. Schäfer, C. A. Schriever, A. Sacchi, E. Capri, J. Biggs, C. Wells, and M. Liess. 2009. SPEAR indicates pesticide effects in streams - Comparative use of species- and family-level biomonitoring data. Environmental Pollution 157:1841–1848.

Bell, M., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Page 211–236 *in* M. Arts, M. Brett, and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, Dordrecht.

Bell, M. V., J. R. Dick, T. R. Anderson, and D. W. Pond. 2007. Application of liposome and stable isotope tracer techniques to study polyunsaturated fatty acid biosynthesis in marine zooplankton. Journal of Plankton Research 29:417–422.

Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity. Ecology Letters 15:365–377.

Benbrook, C. M. 2016. Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28:3.

Bérard, A., and C. Benninghoff. 2001. Pollution-induced community tolerance (PICT) and seasonal variations in the sensitivity of phytoplankton to atrazine in nanocosms. Chemosphere 45:427–437.

Bertness, M. D., and R. M. Callaway. 1994. Positive interactions in communities. Trends in Ecology & Evolution 9:191–193.

Bester, K., H. Hühnerfuss, U. Brockmann, and H. J. Rick. 1995. Biological effects of triazine herbicide contamination on marine phytoplankton. Archives of Environmental Contamination and Toxicology 29:277–283.

Bianchini, A., S. E. G. Martins, and I. F. Barcarolli. 2004. Mechanism of acute copper toxicity in euryhaline crustaceans: implications for the Biotic Ligand Model. International Congress Series 1275:189–194.

Böger, P., B. Matthes, and J. Schmalfuß. 2000. Towards the primary target of chloroacetamides – new findings pave the way. Pest Management Science 56:497–508.

Bolam, S. G., T. F. Fernandes, and M. Huxham. 2002. Diversity, biomass, and ecosystem processes in the marine benthos. Ecological Monographs 72:599–615.

Bopp, L., O. Aumont, P. Cadule, S. Alvain, and M. Gehlen. 2005. Response of diatoms distribution to global warming and potential implications: A global model study. Geophysical Research Letters 32:L19606.

Borer, A. E. T., E. W. Seabloom, J. B. Shurin, K. E. Anderson, C. A. Blanchette, S. D. Cooper, and B. S. Halpern. 2005. What determines the strength of a trophic cascade ? Ecology 86:528–537.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations (with discussion). Journal of the Royal Statistical Society B 26:211–252.

Bracken, M. E. S., and N. H. N. Low. 2012. Realistic losses of rare species disproportionately impact higher trophic levels. Ecology Letters 15:461–467.

Bracken, M. E. S., S. E. Friberg, C. A. Gonzalez-Dorantes, and S. L. Williams. 2008. Functional consequences of realistic biodiversity changes in a marine ecosystem. Proceedings of the National Academy of Sciences of the United States of America 105:924–928.

Brain, P., and R. Cousens. 1989. An equation to describe dose response where there is stimulation of growth at low doses. Weed Research 29:93–96.

Brand, L. E., W. G. Sunda, and R. R. L. Guillard. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. Journal of Experimental Marine Biology and Ecology 96:225–250.

Brett, M. T., C. Mu, A. P. Ballantyne, J. L. Ravet, C. R. Goldman, and D. C. Müller-Navarra. 2006. Daphnia fatty acid composition reflects that of their diet. Limnology and Oceanography. 51:2428–2437.

Brett, M. T., D. C. Muller- Navarra, and S.-K. Park. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. Limnol. Oceanogr. 45:1564–1575.

Brett, M. T., and D. C. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483–499.

Bruckner, C. G., R. Bahulikar, M. Rahalkar, B. Schink, and P. G. Kroth. 2008. Bacteria associated with benthic diatoms from Lake Constance: Phylogeny and influences on diatom growth and secretion of extracellular polymeric substances. Applied and Environmental Microbiology 74:7740–7749.

4357 Bunker, D. E., F. De Clerck, J. C. Bradford, R. K. Colwell, I. Perfecto, O. L. Phillips, M. Sankaran, and
4358 S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. Science
4359 310:1029–1031.

4360 Bruno, J. F., K. E. Boyer, J. E. Duffy, and S. C. Lee. 2008. Relative And Interactive Effects Of Plant And
4361 Grazer Richness In A Benthic Marine Community. Ecology 89:2518–2528.

4362 Bruno, J. F., S. C. Lee, J. S. Kertesz, R. C. Carpenter, Z. T. Long, and J. Emmett Duffy. 2006. Partitioning
4363 the effects of algal species identity and richness on benthic marine primary production. Oikos
4364 115:170–178.

4365 Buchwalter, D. B., D. J. Cain, C. A. Martin, L. Xie, S. N. Luoma, and T. Garland. 2008. Aquatic insect
4366 ecophysiological traits reveal phylogenetically based differences in dissolved cadmium
4367 susceptibility. Proceedings of the National Academy of Sciences of the United States of
4368 America 105:8321–6.

4369 Buffan-Dubau, E., and K. R. Carman. 2000. Diel feeding behavior of meiofauna and their
4370 relationships with microalgal resources. Limnology and Oceanography 45:381–395.

4371 Burns, C. W., M. T. Brett, and M. Schallenberg. 2011. A comparison of the trophic transfer of fatty
4372 acids in freshwater plankton by cladocerans and calanoid copepods. Freshwater Biology
4373 56:889–903.

4374 Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: Functional diversity and
4375 the maintenance of ecological processes and services. Journal of Applied Ecology 48:1079–
4376 1087.

4377 Cagliari, A., R. Margis, F. dos S. Maraschin, A. C. Turchetto-Zolet, G. Loss, and M. Margis-Pinheiro.
4378 2011. Biosynthesis of Triacylglycerols (TAGs) in plants and algae. International Journal of
4379 Plant Biology 2:e10.

4380 Calabrese, E. J. 2008. Hormesis: why it is important to toxicology and toxicologists. Environmental
4381 Toxicology and Chemistry 27:1451–1474.

4382 Calabrese, E. J., and L. A. Baldwin. 1998. Hormesis as a biological hypothesis. Environmental
4383 Health Perspectives 106:357–362.

4384 Cannon, W. B. 1926. Physiological regulation of normal states: some tentative postulates
4385 concerning biological homeostatics. Page 91 *in* A. Petit, editor. A. Charles Richet: ses amis,
4386 ses collègues, ses élèves. Éditions Médicales.

4387 Carafa, R., J. Wollgast, E. Canuti, J. Ligthart, S. Dueri, G. Hanke, S. J. Eisenreich, P. Viaroli, and J. M.

4388 Zaldívar. 2007. Seasonal variations of selected herbicides and related metabolites in water,
4389 sediment, seaweed and clams in the Sacca di Goro coastal lagoon (Northern Adriatic).
4390 Chemosphere 69:1625–1637.

4391 Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. Nature
4392 472:86–89.

4393 Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace,
4394 D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S.
4395 Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–
4396 67.

4397 Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I.
4398 O'Connor, and A. Gonzalez. 2011. The functional role of producer diversity in ecosystems.
4399 American Journal of Botany 98:572–92.

4400 Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem
4401 functioning through interspecific facilitation. Nature 415:426–9.

4402 Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau.
4403 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature
4404 443:989–92.

4405 Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J.
4406 J. Weis. 2007. Impacts of plant diversity on biomass production increase through time
4407 because of species complementarity. Proceedings of the National Academy of Sciences of the
4408 United States of America 104:18123–8.

4409 Carlisle, D. M., and W. H. Clements. 2005. Leaf litter breakdown, microbial respiration and
4410 shredder production in metal-polluted streams. Freshwater Biology 50:380–390.

4411 Carlton, A. N. C. and J. T. 1998. Accelerating invasion Rate in a Highly Invaded Estuary. Science
4412 279:3.

4413 Carroll, I. T., B. J. Cardinale, and R. M. Nisbet. 2011. Niche and fitness differences relate the
4414 maintenance of diversity to ecosystem function. Ecology 92:1157–1165.

4415 Casula, P., A. Wilby, and M. B. Thomas. 2006. Understanding biodiversity effects on prey in multi-
4416 enemy systems. Ecology Letters 9:995–1004.

4417 CBD. 2010. Strategic Plan for Biodiversity 2011-2020, including Aichi Biodiversity Targets.

4418 Convention on Biological Diversity, Montreal, QC.

4419 Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015.
4420 Accelerated modern human – induced species losses: entering the sixth mass extinction.
4421 Sciences Advances 1:1–5.

4422 Cedergreen, N., C. Ritz, and J. C. Streibig. 2005. Improved empirical models describing hormesis.
4423 Environmental Toxicology and Chemistry 24:3166–72.

4424 Cembella, A. D. 2003. Chemical ecology of eukaryotic microalgae in marine ecosystems. Phycologia
4425 42:420–447.

4426 Chaoui, A., S. Mazhoudi, M. H. Ghorbal, and E. ElFerjani. 1997. Cadmium and zinc induction of lipid
4427 peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L).
4428 Plant Science 127:139–147.

4429 Chapin, F. S. I., E. Schulze, and H. Mooney. 1992. Biodiversity and ecosystem processes. Trends in
4430 Ecology and Evolution 7:677–680.

4431 Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S.
4432 Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing
4433 biodiversity. Nature 405:234–42.

4434 Chapin, F. S. I., P. . Matson, and H. A. Mooney. 2002. Principles of terrestrial ecosystem ecology.
4435 Springer, New York.

4436 Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nisbet,
4437 and T. J. Case. 2002. The interaction between predation and competition. Ecology Letters
4438 5:302–315.

4439 Chepurnov, V. A., D. G. Mann, P. Von Dassow, P. Vanormelingen, J. Gillard, D. Inzé, K. Sabbe, and W.
4440 Vyverman. 2008. In search of new tractable diatoms for experimental biology. BioEssays
4441 30:692–702.

4442 Chia, M. A., A. T. Lombardi, M. D. G. G. Melão, and C. C. Parrish. 2013a. Effects of cadmium and
4443 nitrogen on lipid composition of *Chlorella vulgaris* (Trebouxiophyceae, Chlorophyta).
4444 European Journal of Phycology 48:1–11.

4445 Chia, M. A., A. T. Lombardi, M. da G. G. Melao, and C. C. Parrish. 2013b. Lipid composition of
4446 *Chlorella vulgaris* (Trebouxiophyceae) as a function of different cadmium and phosphate
4447 concentrations. Aquatic Toxicology 128–129:171–182.

4448 Chirnside, A. E. M., W. F. Ritter, and M. Radosevich. 2007. Isolation of a selected microbial
4449 consortium from a pesticide-contaminated mix-load site soil capable of degrading the
4450 herbicides atrazine and alachlor. Soil Biology and Biochemistry 39:3056–3065.

4451 Cho, B. C., and F. Azam. 1988. Major role of bacteria in biogeochemical fluxes in the ocean's
4452 interior. Nature 322:441–443.

4453 Choat, J. H., and K. D. Clements. 1998. Vertebrate herbivores in marine and terrestrial
4454 environments: a nutritional ecology perspective. Ecology 29:375–403.

4455 Cid, A., C. Herrero, E. Torres, and J. Abalde. 1995. Copper toxicity on the marine microalga
4456 *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. Aquatic
4457 Toxicology 1.

4458 Clarke, K., and R. Gorley. 2006. PRIMER v6: user manual/ tutorial. PRIMER-E, Plymouth.

4459 Clements, W. H., and J. R. Rohr. 2009. Community responses to contaminants: using basic
4460 ecological principles to predict ecotoxicological effects. Environmental Toxicology and
4461 Chemistry 28:1789.

4462 Cleveland, J. S., M. J. Perry, D. A. Kiefer, and M. C. Talbot. 1989. Maximal quantum yield of
4463 photosynthesis in the northwest Sargasso Sea. Journal of Marine Research 47:869–886.

4464 Cnudde, C., T. Moens, E. Werbrouck, G. Lepoint, D. Van Gansbeke, and M. De Troch. 2015.
4465 Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope
4466 composition and fatty acid profiles. Marine Ecology Progress Series 524:225–239.

4467 Cnudde, C., A. Willems, K. Van Hoorde, W. Vyverman, T. Moens, and M. De Troch. 2011. Effect of
4468 food preservation on the grazing behavior and on the gut flora of the harpacticoid copepod
4469 *Paramphiascella fulvofasciata*. Journal of Experimental Marine Biology and Ecology 407:63–
4470 69.

4471 Cobbett, C., and P. Goldsbrough. 2002. Phytochelatins and metallothioneins: roles in heavy metal
4472 detoxification and homeostasis. Annual Reviews in Plant Biology 53:159–182.

4473 Consalvey, M., R. G. Perkins, D. M. Paterson, and G. J. C. Underwood. 2005. PAM Fluorescence: a
4474 beginners guide for benthic diatomists. Diatom Research 20:1–22.

4475 Coull, B. C. 1990. Are members of the meiofauna food for higher trophic levels? Transactions of
4476 the American Microscopical Society 109:233–246.

4477 Covich, A. P., M. C. Austen, F. Bärlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, O.

4478 Dangles, M. Solan, M. O. Gessner, B. Statzner, and B. Moss. 2004. The role of biodiversity in
4479 the functioning of freshwater and marine benthic ecosystems. BioScience 54:767.

4480 Cowie, G. L. 1996. Digestion and alternation of the biochemical constituents of a diatom
4481 (*Thalassiosira weissflogii*) ingested by an herbivorous zooplankton (*Calanus pacificus*).
4482 Limnology and Oceanography 41:581–594.

4483 Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple
4484 human stressors in marine systems. Ecology Letters 11:1304–1315.

4485 Croft, M. T., A. D. Lawrence, E. Raux-Deery, M. J. Warren, and A. G. Smith. 2005. Algae acquire
4486 vitamin B12 through a symbiotic relationship with bacteria. Nature 438:90–3.

4487 Cross, W. F., J. P. Benstead, A. D. Rosemond, and J. B. Wallace. 2003. Consumer-resource
4488 stoichiometry in detritus-based streams. Ecology Letters 6:721–732.

4489 Dade, W. B., J. D. Davis, P. D. Nichols, A. R. M. Nowell, D. Thistle, M. B. Trexler, and D. C. White. 1990.
4490 Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiology
4491 Journal 8:1–16.

4492 Danovaro, R., M. Scopa, C. Gambi, and S. Fraschetti. 2007. Trophic importance of subtidal metazoan
4493 meiofauna: evidence from in situ exclusion experiments on soft and rocky substrates. Marine
4494 Biology 152:339–350.

4495 Davies, T. W., S. R. Jenkins, R. Kingham, J. Kenworthy, S. J. Hawkins, and J. G. Hiddink. 2011.
4496 Dominance, biomass and extinction resistance determine the consequences of biodiversity
4497 loss for multiple coastal ecosystem processes. PloS ONE 6:e28362.

4498 Debelius, B., J. M. Forja, A. Del Valls, and L. M. Lubián. 2008. Effect of linear alkylbenzene sulfonate
4499 (LAS) and atrazine on marine microalgae. Marine Pollution Bulletin 57:559–568.

4500 Debenest, T., E. Pinelli, M. Coste, J. Silvestre, N. Mazzella, C. Madigou, and F. Delmas. 2009.
4501 Sensitivity of freshwater periphytic diatoms to agricultural herbicides. Aquatic Toxicology
4502 93:11–17.

4503 Debenest, T., J. Silvestre, M. Coste, and E. Pinelli. 2010. Effects of pesticides on freshwater diatoms.
4504 Reviews of Environmental Contamination and Toxicology 203:87–103.

4505 De Brouwer, J. F. C., S. Bjelic, E. M. G. T. De Deckere, and L. J. Stal. 2000. Interplay between biology
4506 and sedimentology in a mudflat (Biezelingse Ham, Westerschelde, The Netherlands).
4507 Continental Shelf Research 20:1159–1177.

4508 De Brouwer, J. F. C. 2002. Dynamics in extracellular carbohydrate production by marine benthic

diatoms. PhD Thesis, Katholieke Universiteit Nijmegen.

De Brouwer, J. F. C., K. Wolfstein, G. K. Ruddy, T. E. R. Jones, and L. J. Stal. 2005. Biogenic stabilization of intertidal sediments: the importance of extracellular polymeric substances produced by benthic diatoms. Microbial Ecology 49:501–512.

De Brouwer, J. F. C., K. Wolfstein, and L. J. Stal. 2002. Physical characterization and diel dynamics of different fractions of extracellular polysaccharides in an axenic culture of a benthic diatom. European Journal of Phycology 37:37–44.

Decho, A. W. 1990. Microbial exopolymer secretions in ocean environments - their role(s) in food webs and marine processes. Oceanography and Marine Biology 78:73–153.

Decho, A. W. 2000. Microbial biofilms in intertidal systems: an overview. Continental Shelf Research 20:1257–1273.

Decho, A. W., and J. W. Fleeger. 1988. Ontogenetic feeding shifts in the meiobenthic harpacticoid copepod *Nitocra lacustris*. Marine Biology 97:191–197.

de Deckere, E. M. G. T., T. J. Tolhurst, and J. F. C. de Brouwer. 2001. Destabilization of cohesive intertidal sediments by infauna. Estuarine, Coastal and Shelf Science 53:665–669.

De Laender, F., C. J. Melian, R. Bindler, P. J. Van den Brink, M. Daam, H. Roussel, J. Juselius, D. Verschuren, and C. R. Janssen. 2014. The contribution of intra- and interspecific tolerance variability to biodiversity changes along toxicity gradients. Ecology Letters 17:72–81.

De Laender, F., J. R. Rohr, R. Ashauer, D. J. Baird, U. Berger, N. Eisenhauer, V. Grimm, U. Hommen, L. Maltby, C. J. Meliàn, F. Pomati, I. Roessink, V. Radchuk, and P. J. van den Brink. 2016. Re-introducing environmental change drivers in biodiversity-ecosystem functioning research. Trends in Ecology and Evolution Evolution 31:905-915.

De Laender, F., K. Soetaert, and J. J. Middelburg. 2010. Inferring chemical effects on carbon flows in aquatic food webs: methodology and case study. Environmental Pollution 158:1775–82.

DeLorenzo, M. E., G. I. Scott, and P. E. Ross. 1999. Effects of the agricultural pesticides atrazine, deethylatrazine, endosulfan, and chlorpyrifos on an estuarine microbial food web. Environmental Toxicology and Chemistry 18:2824–2835.

DeLorenzo, M. E., G. I. Scott, and P. E. Ross. 2001. Toxicity of pesticides to aquatic microorganisms: a review. Environmental Toxicology and Chemistry 20:84–98.

De Troch, M., P. Boeckx, C. Cnudde, D. Van Gansbeke, A. Vanreusel, M. Vincx, and M. Caramujo.

2012a. Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. Marine Ecology Progress Series 465:53–67.

De Troch, M., M. Grego, V. A. Chepurnov, and M. Vincx. 2007. Food patch size, food concentration and grazing efficiency of the harpacticoid *Paramphiascella fulvofasciata* (Crustacea, Copepoda). Journal of Experimental Marine Biology and Ecology 343:210–216.

De Troch, M., J. Mees, and E. Wakwabi. 1998. Diets of abundant fishes from beach seine catches in seagrass beds of a tropical bay (Gazi Bay, Kenya). Belgian Journal of Zoology 128:135–154.

De Troch, M., M. B. Steinarsdóttir, V. Chepurnov, and E. Ólafsson. 2005. Grazing on diatoms by harpacticoid copepods: species-specific density-dependent uptake and microbial gardening. Aquatic Microbial Ecology 39:135–144.

De Troch, M., I. Vergaerde, C. Cnudde, P. Vanormelingen, W. Vyverman, and M. Vincx. 2012b. The taste of diatoms: the role of diatom growth phase characteristics and associated bacteria for benthic copepod grazing. Aquatic Microbial Ecology 67:47–58.

De Troch, M., L. Houthoofd, V. Chepurnov, and A. Vanreusel. 2006. Does sediment grain size affect diatom grazing by harpacticoid copepods? Marine Environmental Research 61:265–277.

Deutschman, D. H. 2001. Design and analysis of biodiversity field experiments. Ecological Research 16:833–843.

Diaz, S., and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem processes: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16:646–655.

Díaz, S., A. J. Symstad, F. Stuart Chapin, D. a. Wardle, and L. F. Huenneke. 2003. Functional diversity revealed by removal experiments. Trends in Ecology & Evolution 18:140–146.

Díaz, S., A. Purvis, J. H. C. Cornelissen, G. M. Mace, M. J. Donoghue, R. M. Ewers, P. Jordano, and W. D. Pearse. 2013. Functional traits, the phylogeny of function, and ecosystem service vulnerability. Ecology and Evolution 3:2958–2975.

Dorigo, U., and C. Leboulanger. 2001. A pulse-amplitude modulated fluorescence-based method for assessing the effects of photosystem II herbicides on freshwater periphyton. Journal of Applied Phycology 13:509–515.

Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296–9.

4570 Downing, A. L., and M. a Leibold. 2002. Ecosystem consequences of species richness and
4571 composition in pond food webs. Nature 416:837–841.

4572 Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for
4573 determination of sugars and related substances. Analytical Chemistry 28:350–356.

4574 Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. Ecology Letters
4575 6:680–687.

4576 Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault, and M. Loreau. 2007. The
4577 functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology
4578 Letters 10:522–38.

4579 Dummermuth, A. L., U. Karsten, K. M. Fisch, G. M. König, and C. Wiencke. 2003. Responses of marine
4580 macroalgae to hydrogen-peroxide stress. Journal of Experimental Marine Biology and
4581 Ecology 289:103–121.

4582 Dunstan, G. A., J. K. Volkman, S. M. Barrett, J. M. Leroi, and S. W. Jeffrey. 1993. Essential
4583 polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry
4584 35:155–161.

4585 Van Duyl, F. C., B. De Winder, A. J. Kop, and U. Wollenzien. 1999. Tidal coupling between
4586 carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal
4587 mudflats. Marine Ecology Progress Series 191:19–32.

4588 Eder, K. 1995. Gas chromatographic analysis of fatty acid methyl esters. Journal of
4589 Chromatography B: Biomedical Sciences and Applications 671:113–131.

4590 Eisenhauer, N., A. D. Barnes, S. Cesarz, D. Craven, O. Ferlian, F. Gottschall, J. Hines, A. Sendek, J.
4591 Siebert, M. P. Thakur, and M. Türke. 2016. Biodiversity-ecosystem function experiments
4592 reveal the mechanisms underlying the consequences of biodiversity change in real world
4593 ecosystems. Journal of Vegetation Science:1–10.

4594 Eklöf, J. S., C. Alsterberg, J. N. Havenhand, K. Sundbäck, H. L. Wood, and L. Gamfeldt. 2012.
4595 Experimental climate change weakens the insurance effect of biodiversity. Ecology Letters
4596 15:864–872.

4597 El-Sheekh, M. M., H. M. Kotkat, and O. H. E. Hammouda. 1994. Effect of atrazine herbicide on
4598 growth, photosynthesis, protein synthesis, and fatty acid composition in the unicellular
4599 green alga *Chlorella kessleri*. Ecotoxicology and Environmental Safety 29:349–358.

4600 Emmerson, M. C., M. Solan, C. Emes, D. M. Paterson, and D. Raffaelli. 2001. Consistent patterns and

the idiosyncratic effects of biodiversity in marine ecosystems. Nature 411:73–7.

EU. 2001. Strategy for a future chemicals policy. Brussels.

European Marine Board. 2013. Linking oceans and human health: a strategic research priority for Europe. Ostend, Belgium.

Fargione, J., D. Tilman, R. Dybzinski, J. H. R. Lambers, C. Clark, W. S. Harpole, J. M. H. Knops, P. B. Reich, and M. Loreau. 2007. From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proceedings of the Royal Society B 274:871–6.

Fedtke, C. 1982. Biochemistry and physiology of herbicide action. Springer, New York.

Fernandes, I., C. Pascoal, and F. Cássio. 2011. Intraspecific traits change biodiversity effects on ecosystem functioning under metal stress. Oecologia 166:1019–1028.

Fernandes, J. C., and F. S. Henriques. 1991. Biochemical, physiological, and structural effects of excess copper in plants. The Botanical Review 57:246–273.

Finkel, Z. V, J. Beardall, K. J. Flynn, A. Quigg, T. A. V Rees, and J. A. Raven. 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. Journal of Plankton Research 32.

Fischer, B. B., F. Pomati, and R. I. L. Eggen. 2013. The toxicity of chemical pollutants in dynamic natural systems: The challenge of integrating environmental factors and biological complexity. Science of the Total Environment 449:253–259.

Fisher, N. S., and D. Frood. 1980. Heavy metals and marine diatoms: Influence of dissolved organic compounds on toxicity and selection for metal tolerance among four species. Marine Biology 59:85–93.

Flameling, I. a., and J. Kromkamp. 1998. Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae. Limnology and Oceanography 43:284–297.

Fleeger, J. W., K. R. Carman, and R. M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment 317:207–233.

Flemming, H.-C., and J. Wingender. 2001. Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. Water Science & Technology 43:1–8.

Fontana, S., J. Jokela, and F. Pomati. 2014. Opportunities and challenges in deriving phytoplankton

4631 diversity measures from individual trait-based data obtained by scanning flow-cytometry.
4632 Frontiers in Microbiology 5:1–12.

4633 Forget-Leray, J., I. Landriau, C. Minier, and F. Leboulenger. 2005. Impact of endocrine toxicants on
4634 survival, development, and reproduction of the estuarine copepod Eurytemora affinis
4635 (Poppe). Ecotoxicology and Environmental Safety 60:288–294.

4636 Forster, R. M., V. Créach, K. Sabbe, W. Vyverman, and L. J. Stal. 2006. Biodiversity-ecosystem
4637 function relationship in microphytobenthic diatoms of the Westerschelde estuary. Marine
4638 Ecology Progress Series 311:191–201.

4639 Fouilland, E. 2012. Biodiversity as a tool for waste phycoremediation and biomass production.
4640 Reviews in Environmental Science and Biotechnology 11:1–4.

4641 Fox, J. W. 2004. Effects of algal and herbivore diversity on the partitioning of biomass within and
4642 among trophic levels. Ecology 85:549–559.

4643 Fox, J. W. 2005. Interpreting the 'selection effect' of biodiversity on ecosystem function. Ecology
4644 Letters 8:846–856.

4645 Fox, J. W. 2006. Using the price equation to partition the effects of biodiversity loss on ecosystem
4646 function. Ecology 87:2687–2696.

4647 Fox, J. W., and W. S. Harpole. 2008. Revealing how species loss affects ecosystem function : the
4648 trait-based price equation partition. Ecology 89:269–279.

4649 Fugère, V., P. Andino, R. Espinosa, F. Anthelme, D. Jacobsen, and O. Dangles. 2012. Testing the
4650 stress-gradient hypothesis with aquatic detritivorous invertebrates: insights for
4651 biodiversity-ecosystem functioning research. The Journal of Animal Ecology 81:1259–67.

4652 Galgani, F., N. Piel, and F. Vincent. 1999. A simple procedure for polymerase chain reaction of the
4653 PSBA gene in algae: Application to the screening of mutations conferring atrazine resistance
4654 and discrimination of natural populations of *Porphyra linearis*. Comparative Biochemistry
4655 and Physiology – Part B: Biochemistry and Molecular Biology 124:363–369.

4656 Galloway, R. E., and L. J. Mets. 1984. Atrazine, bromacil, and diuron resistance in *Chlamydomonas*.
4657 A single non-Mendelian genetic locus controls the structure of the thylakoid binding site.
4658 Plant Physiology 74:469–474.

4659 Gamfeldt, L., H. Hillebrand, and P. R. Jonsson. 2005. Species richness changes across two trophic
4660 levels simultaneously affect prey and consumer biomass. Ecology Letters 8:696–703.

Gamfeldt, L., H. Hillebrand, and P. R. Jonsson. 2008. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. Ecology 89:1223–1231.

Gamfeldt, L., J. S. Lefcheck, J. E. K. Byrnes, B. J. Cardinale, J. E. Duffy, and J. N. Griffin. 2015. Marine biodiversity and ecosystem functioning: what's known and what's next? Oikos 124:252–265.

Gamfeldt, L., T. Snäll, R. Bagchi, M. Jonsson, L. Gustafsson, P. Kjellander, M. C. Ruiz-Jaen, M. Fröberg, J. Stendahl, C. D. Philipson, G. Mikusiński, E. Andersson, B. Westerlund, H. Andrén, F. Moberg, J. Moen, and J. Bengtsson. 2013. Higher levels of multiple ecosystem services are found in forests with more tree species. Nature Communications 4:1340.

Gärdes, A., M. H. Iversen, H.-P. Grossart, U. Passow, and M. S. Ullrich. 2011. Diatom-associated bacteria are required for aggregation of Thalassiosira weissflogii. The ISME journal 5:436–45.

Gaspar, T., T. Franck, B. Bisbis, and C. Kevers. 2002. Concepts in plant stress physiology. Application to plant tissue cultures. Plant Growth Regulation 37:263–286.

Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, J. Liira, T. Tscharntke, C. Winqvist, S. Eggers, R. Bommarco, T. Pärt, V. Bretagnolle, M. Plantegenest, L. W. Clement, C. Dennis, C. Palmer, J. J. Oñate, I. Guerrero, V. Hawro, T. Aavik, C. Thies, A. Flohre, S. Hänke, C. Fischer, P. W. Goedhart, and P. Inchausti. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology 11:97–105.

Gekeler, W., E. Grill, E. L. Winnacker, and M. H. Zenk. 1988. Algae sequester heavy metals via synthesis of phytochelatin complexes. Archives of Microbiology 150:197–202.

Gerbersdorf, S. U., R. Bittner, H. Lubarsky, W. Manz, and D. M. Paterson. 2009a. Microbial assemblages as ecosystem engineers of sediment stability. Journal of Soils and Sediments 9:640–652.

Gerbersdorf, S. U., B. Westrich, and D. M. Paterson. 2009b. Microbial extracellular polymeric substances (EPS) in fresh water sediments. Microbial Ecology 58:334–349.

Gessner, M. O., and J. Hines. 2012. Stress as a modifier of biodiversity effects on ecosystem processes? Journal of Animal Ecology 81:1143–1145.

Giere, O. 2009a. Introduction to meiobenthology. Page 1 *in* O. Giere, editor. Meiobenthology: the mcroscopic motile fauna of aquatic sediments. Springer, Berlin, Heidelberg.

Giere, O. 2009b. Synecological perspectives in meiobenthology. Pages 373–415 *in* O. Giere, editor.

Meiobenthology: the microscopic motile fauna of aquatic sediments. Springer, Berlin, Heidelberg.

Giles, K. L., R. D. Madden, R. Stockland, M. E. Payton, and J. W. Dillwith. 2002. Host plants affect predator fitness via the nutritional value of herbivore prey: Investigation of a plant-aphid-ladybeetle system. BioControl 47:1–21.

Giller, P. S., H. Hillebrand, U.-G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M. T. Monaghan, P. J. Morin, and G. O'Mullan. 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. Oikos 104:423–436.

Gladyshev, M. I., N. N. Sushchik, O. V. Anishchenko, O. N. Makhutova, V. I. Kolmakov, G. S. Kalachova, A. A. Kolmakova, and O. P. Dubovskaya. 2011. Efficiency of transfer of essential polyunsaturated fatty acids versus organic carbon from producers to consumers in a eutrophic reservoir. Oecologia 165:521–531.

Godbold, J. A., M. Solan, and K. Killham. 2009. Consumer and resource diversity effects on marine macroalgal decomposition. Oikos 118:77–86.

Goldstein, D. S., and I. J. Kopin. 2007. Evolution of concepts of stress. Stress 10:109–20.

Gonçalves, A. L., A. V. Lírio, J. Pratas, and C. Canhoto. 2011. Uranium contaminated water does not affect microbial activity but decreases feeding by the shredder *Sericostoma vittatum*. Fundamental and Applied Limnology / Archiv für Hydrobiologie 179:17–25.

Gonzalez, A., B. J. Cardinale, G. R. H. Allington, J. Byrnes, K. A. Endsley, D. G. Brown, D. U. Hooper, F. Isbell, M. I. O'Connor, and M. Loreau. 2016. Estimating local biodiversity change: A critique of papers claiming no net loss of local diversity. Ecology 97:1949–1960.

Graymore, M., F. Stagnitti, and G. Allinson. 2001. Impacts of atrazine in aquatic ecosystems. Environment International 26:483–95.

Griffin, J. N., J. E. K. Byrnes, and B. J. Cardinale. 2013. Effects of predator richness on prey suppression: a meta-analysis. Ecology 94:2180–2187.

Griffin, J. N., V. Méndez, A. F. Johnson, S. R. Jenkins, and A. Foggo. 2009. Functional diversity predicts overyielding effect of species combination on primary productivity. Oikos 118:37–44.

Griffiths, R. I., A. S. Whiteley, G. Anthony, O. Donnell, M. J. Bailey, and A. G. O. Donnell. 2003.

Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69:6961–6968.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary. The American Naturalist 111:1169–1194.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86:902/910.

De Groot, R. S., M. A. Wilson, and R. M. J. Boumans. 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological Economics 41:1–20.

Gross, K., and B. J. Cardinale. 2005. The functional consequences of random vs. ordered species extinctions. Ecology Letters 8:409–418.

Grossart, H.-P. 1999. Interactions between marine bacteria and axenic various conditions in the lab. Aquatic Microbial Ecology 19:1–11.

Grossart, H. P., and H. Ploug. 2001. Microbial degradation of organic carbon and nitrogen on diatom aggregates. Limnol. Oceanogr. 46:267–277.

Guasch, H., N. Ivorra, V. Lehmann, M. Paulsson, M. Real, and S. Sabater. 1998. Community composition and sensitivity of periphyton to atrazine in owing waters: the role of environmental factors. Journal of Applied Phycology 10:203–213.

Guénard, G., P. C. Von Der Ohe, D. De Zwart, P. Legendre, and S. Lek. 2011. Using phylogenetic information to predict species tolerances to toxic chemicals. Ecological Applications 21:3178–3190.

Guerrini, F., A. Mazzotti, L. Boni, and R. Pistocchi. 1998. Bacterial-algal interaction in polysaccharide production. Aquatic Microbial Ecology 15:247–253.

Guillard, R. L. 1975. Culture of Phytoplankton for Feeding Marine Invertebrates. Pages 29–60 *in* W. Smith and M. Chanley, editors. Culture of Marine Invertebrate Animals, Springer. New York.

Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2016. The importance of high-quality algal food sources in stream food webs - current status and future perspectives. Freshwater Biology 61:815–831.

Guschina, I. A., and J. L. Harwood. 2006. Lipids and lipid metabolism in eukaryotic algae. Progress

in Lipid Research 45:160–186.

Guschina, I. A., and J. L. Harwood. 2009. Algal lipids and effect of the environment on their biochemistry. Pages 1–24 *in* M. T. Arts, M. T. Brett, and M. J. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York.

Gustafsson, C., and C. Boström. 2011. Biodiversity influences ecosystem functioning in aquatic angiosperm communities. Oikos 120:1037–1046.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters 12:1029–1039.

Hall, A., A. H. Fielding, and M. Butler. 1979. Mechanisms of copper tolerance in the marine fouling alga *Ectocarpus siliculosus* - Evidence for an exclusion mechanism. Marine Biology 54:195–199.

Hall, L. W., M. C. Ziegenfuss, R. D. Anderson, and D. P. Tierney. 1995. The influence of salinity on the chronic toxicity of atrazine to an estuarine copepod: Implications for development of an estuarine chronic criterion. Archives of Environmental Contamination and Toxicology 28:344–348.

Halpern, B. S., K. A. Selkoe, F. Micheli, and C. V. Kappel. 2007. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conservation Biology 21:1301–1315.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig, M. Spalding, R. Steneck, and R. Watson. 2008. A global map of human impact on marine ecosystems. Science 319:948–953.

Halstead, N. T., T. A. Mcmahon, S. A. Johnson, T. R. Raffel, J. M. Romansic, P. W. Crumrine, and J. R. Rohr. 2014. Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. Ecology Letters 17:932–941.

Hamm, C. E., R. Merkel, O. Springer, and P. Jurkojc. 2003. Architecture and material properties of diatom shells provide effective mechanical protection. Nature 421:841–843.

Hänsch, R., and R. R. Mendel. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Current Opinion in Plant Biology 12:259–266.

Hartig, P., K. Wolfstein, S. Lippemeier, and F. Colijn. 1998a. Photosynthetic activity of natural microphytobenthos populations measured by fluorescence. Marine Ecology Progress Series 166:53–62.

Hartig, P., K. Wolfstein, and S. Lippemeier. 1998b. Photosynthetic activity of natural microphyto-benthos populations measured by fluorescence (PAM) and 14C-tracer methods: a comparison. Marine Ecology Progress Series 166:53–62.

Harwood, J. L., and I. A. Guschina. 2009. The versatility of algae and their lipid metabolism. Biochimie 91:679–684.

Hautier, Y., D. Tilman, F. Isbell, E. W. Seabloom, E. T. Borer, and P. B. Reich. 2015. Anthropogenic environmental changes affect ecosystem stability via biodiversity. Science 348:336–340.

Hawlena, D., and O. J. Schmitz. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. The American Naturalist 176:537–556.

Hayes, T. B., L. L. Anderson, V. R. Beasley, S. R. De Solla, T. Iguchi, H. Ingraham, P. Kestemont, J. Kniewald, Z. Kniewald, V. S. Langlois, E. H. Luque, K. A. McCoy, M. Muñoz-De-Toro, T. Oka, C. A. Oliveira, F. Orton, S. Ruby, M. Suzawa, L. E. Tavera-Mendoza, V. L. Trudeau, A. B. Victor-Costa, and E. Willingham. 2011. Demasculinization and feminization of male gonads by atrazine: Consistent effects across vertebrate classes. Journal of Steroid Biochemistry and Molecular Biology 127:64–73.

Haynes, K. C., and R. L. Guillard. 1974. Growth of vitamin B12-requiring marine diatoms in mixed laboratory cultures with vitamin B12-producing marine bacteria. Journal of Phycology 10:245–252

Haynes, K., T. A. Hofmann, C. J. Smith, A. S. Ball, G. J. C. Underwood, and A. M. Osborn. 2007. Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. Applied and Environmental Microbiology 73:6112–6124..

He, Q., M. D. Bertness, and A. H. Altieri. 2013. Global shifts towards positive species interactions with increasing environmental stress. Ecology Letters 16:695–706.

Hector, A., and R. Bagchi. 2007. Biodiversity and ecosystem multifunctionality. Nature 448:188–90.

Hector, A., T. Bell, J. Connolly, J. Finn, J. W. Fox, L. Kirwan, M. Loreau, J. McLaren, B. Schmid, and A. Weigelt. 2009. The analysis of biodiversity experiments: from pattern towards mechanism. Pages 94–104 *in* S. Naeem, editor. Biodiversity, ecosystem functioning, and human

wellbeing: an ecological and economic perspective. Oxford University Press, Oxford.

Hector, A., J. Joshi, S. Lawler, E. Spehn, and A. Wilby. 2001. Conservation implications of the link between biodiversity and ecosystem functioning. Oecologia 129:624–628.

Hector, A., B. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. Finn, H. Freitas, P. Giller, J. Good, and R. Harris. 2000. No consistent effect of plant diversity on productivity: response. Science 289:1255a.

Hector, A., C. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. a Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, J. Joshi, A. Jumpponen, C. Körner, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, J. S. Pereira, A. Prinz, D. J. Read, M. Scherer-lorenzen, E. D. Schulze, A.-. S. D. Siamantziouras, E. M. Spehn, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 1999. Plant diversity and productivity experiments in European grasslands. Science 286:1123–1127.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1999. 'Sampling effect', a problem in biodiversity manipulation? A reply to David A. Wardle. Oikos 87:408–410.

Hellebust, J. A., and J. Lewin. 1977. Heterotrophy. Pages 169–197 *in* D. Werner, editor. The biology of diatoms. Botanical Monographs. University of California Press, Berkeley.

Heuner, M., A. Silinski, J. Schoelynck, T. J. Bouma, S. Puijalon, P. Troch, E. Fuchs, B. Schröder, U. Schröder, P. Meire, and S. Temmerman. 2015. Ecosystem engineering by plants on wave-exposed intertidal flats is governed by relationships between effect and response traits. PLoS ONE 10:1–18.

Hicks, N., M. T. Bulling, M. Solan, D. Raffaelli, P. C. L. White, and D. M. Paterson. 2011. Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system. BMC Ecology 11:7.

Hiddink, J. G., T. Wynter Davies, M. Perkins, M. Machairopoulou, and S. P. Neill. 2009. Context dependency of relationships between biodiversity and ecosystem functioning is different for multiple ecosystem functions. Oikos 118:1892–1900.

Hillebrand, H., D. M. Bennett, and M. W. Cadotte. 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. Ecology 89:1510–1520.

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 424:403–424.

Hillebrand, H., and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. Ecology Letters 12:1405–19.

Hillebrand, H., B. Worm, and H. K. Lotze. 2000. Marine microbenthic community structure related by nitrogen loading and grazing pressure. Marine Ecology Progress Series 204:27–38.

Hobbs, R. J. 1992. Is biodiversity important for ecosystem functioning - implications for research and management. Page 211- Biodiversity of mediterranean ecosystems in Australia. Perth, Australia.

Hodapp, D., H. Hillebrand, B. Blasius, and A. B. Ryabov. 2016. Environmental and trait variability constrain community structure and the biodiversity-productivity relationship. Ecology 97:1463–1474.

Hogsden, K. L., and J. S. Harding. 2012. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science 31:108–120.

Hooper, D., F. I. Chapin, J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. Lawton, D. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. Symstad, J. Vandermeer, and D. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. a Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105–8.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346--363.

Hoyt, M., J. W. Fleeger, R. Siebeling, and R. J. Feller. 2000. Serological estimation of prey-protein gut-residence time and quantification of mean size for grass shrimp consuming meiofaunal copepods. Journal of Experimental Marine Biology and Ecology 248:105–119.

Hu, Z., Y. Zheng, F. Yan, B. Xiao, and S. Liu. 2013. Bio-oil production through pyrolysis of blue-green algae blooms (BGAB): product distribution and bio-oil characterization. Energy 52:119–125.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters 11:609–623.

Hughes, A. R., R. J. Best, and J. J. Stachowicz. 2010. Genotypic diversity and grazer identity interactively influence seagrass and grazer biomass. Marine Ecology Progress Series 403:43–51.

Hünken, M., J. Harder, and G. O. Kirst. 2008. Epiphytic bacteria on the Antarctic ice diatom *Amphiprora kufferathii* Manguin cleave hydrogen peroxide produced during algal photosynthesis. Plant Biology 10:519–526.

Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evalutating the ecosystem function of biodiverstiy. Oecologia 110:449–460.

Huston, M. A., L. W. Aarssen, M. P. Austin, B. S. Cade, J. D. Fridlez, E. Garnier, J. P. Grime, J. Hodgson, W. K. Lauenroth, K. Thompson, J. H. Vandermeer, and D. A. Wardle. 2000. No consistent effect of plant diversity on productivity. Science 289:1255a–1255.

Ianora, A., and A. Miralto. 2010. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: A review. Ecotoxicology 19:493–511.

Imhoff, M., L. Bounoua, T. Ricketts, C. Loucks, R. Harriss, and W. T. Lawrence. 2004. Global patterns in human consumption of net primary production. Nature 429:870–873.

InVEST. 2016. InVEST - Integrated Valuation of Ecosystem Services and Tradeoffs: http://www.naturalcapitalproject.org/invest/. Natural Capital Project.

IPBES. 2016. Scenarios and models of biodiversity and ecosystem services - summary for policymakers. Bonn, Germany.

Isbell, F. I., H. W. Polley, and B. J. Wilsey. 2009. Biodiversity, productivity and the temporal stability of productivity: Patterns and processes. Ecology Letters 12:443–451.

Isbell, F., V. Calcagno, A. Hector, J. Connolly, W. S. Harpole, P. B. Reich, M. Scherer-Lorenzen, B. Schmid, D. Tilman, J. van Ruijven, A. Weigelt, B. J. Wilsey, E. S. Zavaleta, and M. Loreau. 2011. High plant diversity is needed to maintain ecosystem services. Nature 477:199–202.

Islam, M. S., and M. Tanaka. 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Marine Pollution Bulletin 48:624–649.

Ives, A. R., and B. J. Cardinale. 2004. Food-web interactions govern the resistance of communities after non-random extinctions. Nature 429:174–177.

Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. Science 58:58–63.

Janssen, C., P. Roose, H. DE De Brabander, M. Vincx, and J. Mees. 2010. Integrated risk assessment and monitoring of micropollutants in the belgian coastal zone – INRAM - Final Report Phase 1. Belgian Science Policy:53.

4905 Jiang, L. 2007. Negative selection effects suppress relationships between bacterial diversity and
4906 ecosystem functioning. Ecology 88:1075–1085.

4907 Jiang, L., Z. Pu, and D. R. Nemergut. 2008. On the importance of the negative selection effect for the
4908 relationship between biodiversity and ecosystem functioning. Oikos 117:488–493.

4909 Jodice, P. G. R., D. D. Roby, K. R. Turco, R. M. Suryan, D. B. Irons, J. F. Piatt, M. T. Shultz, D. G.
4910 Roseneau, A. B. Kettle, and J. A. Anthony. 2006. Assessing the nutritional stress hypothesis:
4911 Relative influence of diet quantity and quality on seabird productivity. Marine Ecology
4912 Progress Series 325:267–279.

4913 Johnston, E. L., and D. A. Roberts. 2009. Contaminants reduce the richness and evenness of marine
4914 communities: a review and meta-analysis. Environmental Pollution 157:1745–52.

4915 Jonsson, M., O. Dangles, B. Malmqvist, and F. Guérold. 2002. Simulating species loss following
4916 perturbation: assessing the effects on process rates. Proceedings of the Royal Society B
4917 269:1047–52.

4918 Jung, S. W., B. H. Kim, T. Katano, D. S. Kong, and M. S. Han. 2008. Pseudomonas fluorescens
4919 HYK0210-SK09 offers species-specific biological control of winter algal blooms caused by
4920 freshwater diatom *Stephanodiscus hantzschii*. Journal of Applied Microbiology 105:186–195.

4921 Jüttner, I., A. Peither, J. P. Lay, A. Kettrup, and S. J. Ormerod. 1995. An outdoor mesocosm study to
4922 assess ecotoxicological effects of atrazine on a natural plankton community. Archives of
4923 Environmental Contamination and Toxicology 29:435–441.

4924 Kaczmarska, I., J. M. Ehrman, S. S. Bates, D. H. Green, C. Léger, and J. Harris. 2005. Diversity and
4925 distribution of epibiotic bacteria on Pseudo-nitzschia multiseries (Bacillariophyceae) in culture,
4926 and comparison with those on diatoms in native seawater. Harmful Algae 4:725–741.

4927 Kainz, M. J., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web
4928 and their ecological role for higher trophic levels. Limnology and Oceanography 49:1784–
4929 1793.

4930 Kareiva, P., H. Tallis, T. H. Ricketts, G. C. Daily, and S. Polasky. 2011. Natural capital: theory and
4931 practice of mapping ecosystem services. Oxford University Press, Oxford.

4932 Karlson, A. M. L., F. J. A. Nascimento, J. Näslund, and R. Elmgren. 2010. Higher diversity of deposit-
4933 feeding macrofauna enhances phytodetritus processing. Ecology 91:1414–1423.

4934 Karp, D. S., H. V. Moeller, and L. O. Frishkoff. 2013. Nonrandom extinction patterns can modulate

pest control service decline. Ecological Applications 23:840–849.

Kasai, F., and T. Hanazato. 1995. Effects of the triazine herbicide, simetryn, on freshwater plankton communities in experimental ponds. Environmental Pollution 89:197–202.

Kearns, P. J., N. B. Weston, J. L. Bowen, T. Živković, and M. A. Vile. 2016. Tidal freshwater marshes harbor phylogenetically unique clades of sulfate reducers that are resistant to climate-change-induced salinity intrusion. Estuaries and Coasts 39:981–991.

Kennish, M. J. 1996. Practical handbook of estuarine and marine pollution. CRC Press, Boca Raton, Florida, USA.

Kirwan, L., A. Lüscher, M. T. Sebastià, J. a. Finn, R. P. Collins, C. Porqueddu, A. Helgadottir, O. H. Baadshaug, C. Brophy, C. Coran, S. Dalmannsdóttir, I. Delgado, A. Elgersma, M. Fothergill, B. E. Frankow-Lindberg, P. Golinski, P. Grieu, a. M. Gustavsson, M. Höglind, O. Huguenin-Elie, C. Iliadis, M. Jørgensen, Z. Kadziuliene, T. Karyotis, T. Lunnan, M. Malengier, S. Maltoni, V. Meyer, D. Nyfeler, P. Nykanen-Kurki, J. Parente, H. J. Smit, U. Thumm, and J. Connolly. 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. Journal of Ecology 95:530–539.

Kitaysky, A. S., E. V Kitaiskaia, J. F. Piatt, and J. C. Wingfield. 2006. A mechanistic link between chick diet and decline in seabirds? Proceedings. Biological sciences / The Royal Society 273:445–450.

Kloareg, B., and R. S. Quatrano. 1988. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. Pages 259–315 *in* H. Barnes and M. Barnes, editors. Oceanography and Marine Biology - An Annual Review. Aberdeen University Press, Aberdeen.

Klumpp, K., and J. F. Soussana. 2009. Using functional traits to predict grassland ecosystem change: A mathematical test of the response-and-effect trait approach. Global Change Biology 15:2921–2934.

Knauert, S. 2008. Toxicity of pesticides and their mixture to primary producers.

Knauert, S., and K. Knauer. 2008. The role of reactive oxygen species in copper toxicity to two freshwater green algae. Journal of Phycology 44:311–319.

Koelmans, A. A., A. Van der Heijde, L. M. Knijgg, and R. H. Aalderink. 2001. Integrated modelling of eutrophication and organic contaminant fate & effects in aquatic ecosystems. A review. Water Research 35:3517–3536.

Koide, R. T., C. Fernandez, and G. Malcolm. 2014. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. New Phytologist 201:433–439.

Koricheva, J., C. P. H. Mulder, B. Schmid, J. Joshi, and K. Huss-Danell. 2000. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. Oecologia 125:271–282.

Körner, C. 2003. Limitation and stress - always or never? Journal of Vegetation Science 14:141–143.

Koski, M., W. K. Breteler, and N. Schogt. 1998. Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Marine Ecology Progress Series 170:169–187.

Kromkamp, J., C. Barranguet, and J. Peene. 1998. Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. Marine Ecology Progress Series 162:45–55.

Kromkamp, J., and J. Peene. 1999. Estimation of phytoplankton photosynthesis and nutrient limitation in the Eastern Scheldt estuary using variable fluorescence. Aquatic Ecology 33:101–104.

Kroon, B. M. A. 1994. Variability of Photosystem II quantum yield and related processes in *Chlorella pyrenoidosa* (Chlorophyta) acclimated to an oscillating light egime simulating a mixed photic zone. Journal of Phycology 30:841–852.

Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lürling, C. S. Reynolds, and M. Scheffer. 2010. A morphological classification capturing functional variation in phytoplankton. Freshwater Biology 55:614–627.

Lage, O. M., A. M. Parente, H. M. V. M. Soares, M. T. S. D. Vasconcelos, and R. Salema. 1994. Some effects of copper on the dinoflagellates *Amphidinium carterae* and *Prorocentrum micans* in batch culture. European Journal of Phycology 29:253–260.

Lahive, E., J. O' Halloran, and M. A. K. Jansen. 2011. Differential sensitivity of four Lemnaceae species to zinc sulphate. Environmental and Experimental Botany 71:25–33.

Larigauderie, A., and H. A. Mooney. 2010. The Intergovernmental science-policy Platform on Biodiversity and Ecosystem Services: moving a step closer to an IPCC-like mechanism for biodiversity. Current Opinion in Environmental Sustainability 2:9–14.

4997 Larras, F., A. Bouchez, F. Rimet, and B. Montuelle. 2012. Using bioassays and species sensitivity
4998 distributions to assess herbicide toxicity towards benthic diatoms. PLoS ONE 7:e44458.

4999 Larras, F., V. Gregorio, A. Bouchez, B. Montuelle, and N. Chèvre. 2016. Comparison of specific
5000 versus literature species sensitivity distributions for herbicides risk assessment.
5001 Environmental Science and Pollution Research 23:3042–3052.

5002 Larras, F., F. Keck, B. Montuelle, F. Rimet, and A. Bouchez. 2014. Linking diatom sensitivity to
5003 herbicides to phylogeny: A step forward for biomonitoring? Environmental Science &
5004 Technology 48:1921–1930.

5005 Larsen, T. H., N. M. Williams, and C. Kremen. 2005. Extinction order and altered community
5006 structure rapidly disrupt ecosystem functioning. Ecology Letters 8:538–47.

5007 Lau, D. C. P., K. M. Y. Leung, and D. Dudgeon. 2009. Evidence of rapid shifts in the trophic base of
5008 lotic predators using experimental dietary manipulations and assimilation-based analyses.
5009 Oecologia 159:767–776.

5010 Lau, D., K. Leung, and D. Dudgeon. 2009. Are autochthonous foods more important than
5011 allochthonous resources to benthic consumers in tropical headwater streams? Journal of the
5012 North American Benthological Society 28:426–439.

5013 Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem
5014 functioning from plant traits: revisiting the Holy Grail. Functional Ecology 16:545–556.

5015 Lavorel, S., J. Storkey, R. D. Bardgett, F. De Bello, M. P. Berg, X. Le Roux, M. Moretti, C. Mulder, R. J.
5016 Pakeman, S. D??az, and R. Harrington. 2013. A novel framework for linking functional
5017 diversity of plants with other trophic levels for the quantification of ecosystem services.
5018 Journal of Vegetation Science 24:942–948.

5019 Lawler, J. J., J. E. Aukema, J. B. Grant, B. S. Halpern, P. Kareiva, C. R. Nelson, K. Ohleth, J. D. Olden, M.
5020 A. Schlaepfer, B. R. Silliman, and P. Zaradic. 2006. Conservation Science: a 20-year report
5021 card. Frontiers in Ecology and the Environment 4:473–480.

5022 Lefcheck, J. S., J. E. K. Byrnes, F. Isbell, L. Gamfeldt, J. N. Griffin, N. Eisenhauer, M. J. S. Hensel, A.
5023 Hector, B. J. Cardinale, and J. E. Duffy. 2015. Biodiversity enhances ecosystem
5024 multifunctionality across trophic levels and habitats. Nature Communications 6:6936.

5025 Leflaive, J., and L. Ten-Hage. 2007. Algal and cyanobacterial secondary metabolites in freshwaters:
5026 A comparison of allelopathic compounds and toxins. Freshwater Biology 52:199–214.

5027 Legrand, H., O. Herlory, J. M. Guarini, G. F. Blanchard, and P. Richard. 2006. Inhibition of

microphytobenthic photosynthesis by the herbicides atrazine and diuron. Cahiers de Biologie Marine 47:39–45.

Lennon, J. T., and B. K. Lehmkuhl. 2016. A trait-based approach to bacterial biofilms in soil. Environmental Microbiology 18:2732–2742.

Lepš, J. 2004. What do the biodiversity experiments tell us about consequences of plant species loss in the real world? Basic and Applied Ecology 5:529–534.

Levy, J. L., J. L. Stauber, and D. F. Jolley. 2007. Sensitivity of marine microalgae to copper: The effect of biotic factors on copper adsorption and toxicity. Science of the Total Environment 387:141–154.

Lewin, J., and J. A. Hellebust. 1975. Heterotrophic nutrition of the marine pennate diatom *Nitzschia angularis* var. *affinis*. Canadian Journal of Microbiology 21:1335–1342.

Lewis, W. M. 1984. The diatom sex clock and its evolutionary significance. The American Naturalist 123:73–80.

Li, M., C. Hu, Q. Zhu, L. Chen, Z. Kong, and Z. Liu. 2006. Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga *Pavlova viridis* (Prymnesiophyceae). Chemosphere 62:565–572.

Lichtenthaler, H. K. 1996. Vegetation Stress: An Introduction to the Stress Concept in Plants. Plant physiology 148:4–14.

Lichtenthaler, H. K. 1998. The stress concept in plants: an introduction. Annals of the New York Academy of Sciences 851:187–198.

Liebig, M., G. Schmidt, D. Bontje, B. W. Kooi, G. Streck, W. Traunspurger, and T. Knacker. 2008. Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor microcosm. Aquatic Toxicology 88:102–110.

Liess, M., and P. C. Von Der Ohe. 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry 24:954–965.

Litchman, E., and C. A. Klausmeier. 2008. Trait-based community ecology of phytoplankton. Annual Review of Ecology, Evolution, and Systematics 39:615–639.

Litchman, E., C. A. Klausmeier, O. M. Schofield, and P. G. Falkowski. 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. Ecology Letters 10:1170–1181.

5058 Litchman, E., P. de Tezanos Pinto, K. F. Edwards, C. A. Klausmeier, C. T. Kremer, and M. K. Thomas.
5059 2015. Global biogeochemical impacts of phytoplankton: A trait-based perspective. Journal of
5060 Ecology 103:1384–1396.

5061 Litzow, M. a, K. M. Bailey, F. G. Prahl, and R. Heintz. 2006. Climate regime shifts and reorganization
5062 of fish communities: the essential fatty acid limitation hypothesis. Marine Ecology Progress
5063 Series 315:1–11.

5064 Liu, H., and E. J. Buskey. 2000. Hypersalinity enhances the production of Extracellular Polymeric
5065 Substance (EPS) in the Texas brown tide alga, *Aureoumbra lagunsensis* (Pelagophyceae).
5066 Journal of Phycology 36:71–77.

5067 Lockert, C. K., K. D. Hoagland, and B. D. Siegfried. 2006. Comparative sensitivity of freshwater algae
5068 to atrazine. Bulletin of Environmental Contamination and Toxicology 76:73–79.

5069 Long, Z. T., S. R. Fegley, and C. H. Peterson. 2013. Fertilization and plant diversity accelerate
5070 primary succession and restoration of dune communities. Plant Ecology 214:1419–1429.

5071 Loos, R., B. M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, and G. Bidoglio. 2009. EU-wide survey
5072 of polar organic persistent pollutants in European river waters. Environmental Pollution
5073 157:561–568.

5074 Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos
5075 91:3–17.

5076 Loreau, M. 2010. Linking biodiversity and ecosystems: towards a unifying ecological theory.
5077 Philosophical transactions of the Royal Society of London. Series B, Biological Sciences
5078 365:49–60.

5079 Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity
5080 experiments. Nature 412:72–6.

5081 Loreau, M., S. Naeem, and P. Inchausti. 2001a. Biodiversity and ecosystem functioning: the
5082 emergence of a synthetic ecological framework. Pages 3–11 *in* Biodiversity and ecosystem
5083 functioning: synthesis and perspectives. Oxford University Press, Oxford.

5084 Loreau, M., S. Naeem, and P. Inchausti. 2002. Biodiversity and ecosystem functioning. Synthesis
5085 and perspectives. Oxford University Press, Oxford.

5086 Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D.
5087 Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001b. Biodiversity and ecosystem

functioning : current knowledge and future challenges. Science 294:804–808.

Lotze, H. K., H. S. Lenihan, B. J. Bourque, R. H. Bradbury, R. G. Cooke, M. C. Kay, S. M. Kidwell, M. X. Kirby, C. H. Peterson, J. B. C. Jackson, and M. Bay. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809.

Lubarsky, H. V., C. Hubas, M. Chocholek, F. Larson, W. Manz, D. M. Paterson, and S. U. Gerbersdorf. 2010. The Stabilisation Potential of Individual and Mixed Assemblages of Natural Bacteria and Microalgae. PLoS ONE 5.

Lubchenco, J., A. M. Olson, L. B. Brubaker, S. R. Carpenter, M. M. Holland, S. P. Hubbell, S. A. Levin, J. A. Macmahon, A. Pamela, J. M. Melillo, H. A. Mooney, C. H. Peterson, H. R. Pulliam, A. Leslie, P. J. Regal, and P. G. Risser. 1991. The Sustainable biosphere initiative: an ecological research agenda. Ecology 72:371–412.

Mace, G. M., K. Norris, and A. H. Fitter. 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends in Ecology and Evolution 27:19–25.

Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97:199–205.

Maestre, F. T., M. a Bowker, C. Escolar, M. D. Puche, S. Soliveres, S. Maltez-Mouro, P. García-Palacios, A. P. Castillo-Monroy, I. Martínez, and A. Escudero. 2010. Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities. Philosophical Transactions of the Royal Society of London. Series B, Biological sciences 365:2057–2070.

Maestre, F. T., J. L. Quero, N. J. Gotelli, A. Escudero, V. Ochoa, M. Delgado-Baquerizo, M. García-Gómez, M. a Bowker, S. Soliveres, C. Escolar, P. García-Palacios, M. Berdugo, E. Valencia, B. Gozalo, A. Gallardo, L. Aguilera, T. Arredondo, J. Blones, B. Boeken, D. Bran, A. a Conceição, O. Cabrera, M. Chaieb, M. Derak, D. J. Eldridge, C. I. Espinosa, A. Florentino, J. Gaitán, M. G. Gatica, W. Ghiloufi, S. Gómez-González, J. R. Gutiérrez, R. M. Hernández, X. Huang, E. Huber-Sannwald, M. Jankju, M. Miriti, J. Monerris, R. L. Mau, E. Morici, K. Naseri, A. Ospina, V. Polo, A. Prina, E. Pucheta, D. a Ramírez-Collantes, R. Romão, M. Tighe, C. Torres-Díaz, J. Val, J. P. Veiga, D. Wang, and E. Zaady. 2012. Plant species richness and ecosystem multifunctionality in global drylands. Science 335:214–8.

Magnusson, M., K. Heimann, and A. P. Negri. 2008. Comparative effects of herbicides on photosynthesis and growth of tropical estuarine microalgae. Marine Pollution Bulletin

56:1545–1552.

Malaj, E., P. C. von der Ohe, M. Grote, R. Kühne, C. P. Mondy, P. Usseglio-Polatera, W. Brack, and R. B. Schäfer. 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proceedings of the National Academy of Sciences of the United States of America 111:9549–54.

Malmqvist, B., and S. Rundle. 2002. Threats to the running water ecosystems of the world. Environmental Conservation 29:134–153.

Manimaran, K., P. Karthikeyan, S. Ashokkumar, V. A. Prabu, and P. Sampathkumar. 2012. Effect of copper on growth and enzyme activities of marine diatom, *Odontella mobiliensis*. Bulletin of Environmental Contamination and Toxicology 88:30–37.

Manzoni, S., R. B. Jackson, J. a Trofymow, and A. Porporato. 2008. The global stoichiometry of litter nitrogen mineralization. Science 321:684–686.

Martin-Jézéquel, V., M. Hildebrand, and M. A. Brzezinski. 2000. Silicon metabolism in diatoms: implications for growth. Journal of Phycology 36:821–840.

Martín-López, B., C. Montes, and J. Benayas. 2007. The non-economic motives behind the willingness to pay for biodiversity conservation. Biological Conservation 139:67–82.

Masmoudi, S., N. Nguyen-Deroche, A. Caruso, H. Ayadi, A. Morant-Manceau, G. Tremblin, M. Bertrand, and B. Schoefs. 2013. Cadmium, copper, sodium and zinc effects on diatoms: from heaven to hell — a Review. Cryptogamie, Algologie 34:185–225.

Mason, C. F., G. J. C. Underwood, N. R. Baker, P. A. Davey, I. Davidson, A. Hanlon, S. P. Long, K. Oxborough, D. M. Paterson, and A. Watson. 2003. The role of herbicides in the erosion of salt marshes in eastern England. Environmental Pollution 122:41–49.

Mayali, X., and F. Azam. 2004. Algicidal bacteria in the sea and their impact on algal blooms. Journal of Eukaryotic Microbiology 51:139–144.

McCall, J. N., and J. W. Fleeger. 1995. Predation by juvenile fish on hyperbenthic meiofauna: a review with data on post-larval *Leiostomus xanthurus*. Vie et milieu 45:61–73.

McGill, B. J., M. Dornelas, N. J. Gotelli, and A. E. Magurran. 2015. Fifteen forms of biodiversity trend in the anthropocene. Trends in Ecology and Evolution 30:104.

McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem predictability. Nature 390:162–165.

McIntyre, P. B., L. E. Jones, A. S. Flecker, and M. J. Vanni. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. Proceedings of the National Academy of Sciences 104:4461–4466.

McKew, B. A., J. D. Taylor, T. J. McGenity, and G. J. C. Underwood. 2011. Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. The ISME journal 5:30–41.

McKinney, M. L. 1997. Extinction Vulnerability and Selectivity: Combining ecological and paleontological views. Annual Review of Ecology and Systematics 28:495–516.

McLachlan, D. H., C. Brownlee, A. R. Taylor, R. J. Geider, and G. J. C. Underwood. 2009. Light-induced motile responses of the estuarine benthic diatoms *Navicula perminuta* and *Cylindrotheca closterium* (bacillariophyceae). Journal of Phycology 45:592–599.

McMahon, T. A., N. T. Halstead, S. Johnson, T. R. Raffel, J. M. Romansic, P. W. Crumrine, and J. R. Rohr. 2012. Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services. Ecology Letters 15:714–722.

McQuoid, M. R., and L. A. Hobson. 1996. Diatom resting stages. Journal of Phycology 32:889–902.

McWilliam, R. A., and D. J. Baird. 2002. Postexposure feeding depression: a new toxicity endpoint for use in laboratory studies with *Daphnia magna*. Environmental Toxicology and Chemistry 21:1198–1205.

Mehta, S., and J. Gaur. 1999. Heavy-metal-induced proline accumulation and its role in ameliorating metal toxicitiy in Chlorella vulgaris. New Phytologist 143:253–259.

Mensens, C., F. De Laender, C. R. Janssen, K. Sabbe, and M. De Troch. 2015. Stressor-induced biodiversity gradients: revisiting biodiversity-ecosystem functioning relationships. Oikos 124:677–684.

Miao, A.-J., W.-X. Wang, and P. Juneau. 2005. Comparison of Cd, Cu, and Zn toxic effects on four marine phytoplankton by pulse-amplitude-modulated fluorometry. Environmental Toxicology and Chemistry 24:2603.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: biodiversity synthesis. Washington DC.

Millward, R. N., and A. Grant. 2000. Pollution-induced tolerance to copper of nematode communities in the severely contaminated restronguet creek and adjacent estuaries, Cornwall, United Kingdom. Environmental Toxicology and Chemistry 19:454–461.

Moens, T., C. Luyten, J. J. Middelburg, P. M. J. Herman, and M. Vincx. 2002. Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Marine Ecology Progress Series 234:127–137.

Moens, T., A. M. Vafeiadou, E. De Geyter, P. Vanormelingen, K. Sabbe, and M. De Troch. 2014. Diatom feeding across trophic guilds in tidal flat nematodes, and the importance of diatom cell size. Journal of Sea Research 92:125–133.

Moens, T., and M. Vincx. 1997. Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the UK 77:211–227.

Molnar, J. L., R. L. Gamboa, C. Revenga, and M. D. Spalding. 2008. Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:485–492.

Montagna, P. A., G. F. Blanchard, and A. Dinet. 1995. Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. Journal of Experimental Marine Biology and Ecology 185:149–165.

Mooney, H. A. 2002. The debate on the role of biodiversity in ecosystem functioning. Pages 12–17 *in* M. Loreau, S. Naeem, and P. Inchausti, editors. Biodiversity and ecosystem functioning. Oxford University Press, Oxford.

Moran, X. A. G., A. Lopez-Urrutia, A. Calvo-Diaz, and W. K. W. Li. 2010. Increasing importance of small phytoplankton in a warmer ocean. Global Change Biology 16:1137–1144.

Morelli, E., and G. Scarano. 2004. Copper-induced changes of non-protein thiols and antioxidant enzymes in the marine microalga *Phaeodactylum tricornutum*. Plant Science 167:289–296.

Morgan-Kiss, R. M., J. C. Priscu, T. Pocock, L. Gudynaite-Savitch, and N. P. A. Huner. 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiology and Molecular Biology Reviews 70:222–252.

Morin, P. J., and J. McGrady-Steed. 2004. Biodiversity and ecosystem functioning in aquatic microbial systems: a new analysis of temporal variation and species richness-predictability relations. Oikos 104:458–466.

Mulder, C. P., D. D. Uliassi, and D. F. Doak. 2001. Physical stress and diversity-productivity relationships: the role of positive interactions. Proceedings of the National Academy of Sciences of the United States of America 98:6704–6708.

5211 Mullan Crain, C., and M. D. Bertness. 2006. Ecosystem engineering across environmental
5212 gradients: Implications for conservation and management. Bioscience 56:211–218.

5213 Müller-Navarra, D. C. 1995. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in
5214 nature. Archiv für Hydrobiologie 3:297–307.

5215 Müller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty
5216 acid predicts carbon transfer between primary producers and consumers. Nature 403:74–
5217 77.

5218 Murray-Gulde, C. L., J. E. Heatley, A. L. Schwartzman, and J. H. Rodgers. 2002. Algicidal effectiveness
5219 of Clearigate, Cutrine-Plus, and copper sulfate and margins of safety associated with their
5220 use. Archives of Environmental Contamination and Toxicology 43:19–27.

5221 Myklestad, S., O. Holmhansen, K. M. Varum, and B. E. Volcani. 1989. Rate of release of extracellular
5222 amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*. Journal of
5223 Plankton Research 11:763–773.

5224 Naeem, S., and S. Li. 1997. Biodiversity enhances ecosystem reliability. Nature 390:507–510.

5225 Naeem, S. 1999. Ecology - power behind diversity's throne. Nature 401:653–654.

5226 Naeem, S., D. R. Hahn, and G. Schuurman. 2000. Producer – decomposer co-dependency influences
5227 biodiversity effects. Nature 403:762–764.

5228 Naeem, S. 2008. Advancing realism in biodiversity research. Trends in Ecology and Evolution
5229 23:414–6.

5230 Naeem, S., D. E. Bunker, A. Hector, M. Loreau, and C. Perrings. 2009. Biodiversity, ecosystem
5231 functioning, and human wellbeing: an ecological and economic perspective. Oxford
5232 University Press, Oxford.

5233 Naeem, S., J. E. Duffy, and E. Zavaleta. 2012. The functions of biological diversity in an age of
5234 extinction. Science 336:1401–1406.

5235 Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining
5236 biodiversity can alter the performance of ecosystems. Nature 368:734–736.

5237 Naeem, S., and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning:
5238 deriving solutions to a seemingly insormountable problem. Ecology Letters 6:567–579.

5239 Naidoo, R., and T. H. Ricketts. 2006. Mapping the economic costs and benefits of conservation.
5240 PLoS Biology 4:2153–2164.

5241 Nanton, D. A., and J. D. Castell. 1998. The effects of dietary fatty acids on the fatty acid composition
5242 of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae.
5243 Aquaculture 163:251–261.

5244 Neumann-Lee, L. A. 2016. Anthropogenic and natural stressors and their effect on immunity,
5245 reproduction and the stress response. Utah State University.

5246 Niquil, N., G. Kerleguer, D. Leguerrier, P. Richard, H. Legrand, C. Dupuy, P. Y. Pascal, and C. Bacher.
5247 2006. How would the loss of production due to an herbicide have repercussions in the food
5248 web of an intertidal mudflat? Sensitivity analysis of an inverse model for Brouage mudflat,
5249 Marennes-Oléron Bay, France. Cahiers de Biologie Marine 47:63–71.

5250 Nödler, K., T. Licha, and D. Voutsa. 2013. Twenty years later - Atrazine concentrations in selected
5251 coastal waters of the Mediterranean and the Baltic Sea. Marine Pollution Bulletin 70:112–
5252 118.

5253 Noppe, H., A. Ghekiere, T. Verslycke, E. De Wulf, K. Verheyden, E. Monteyne, K. Polfliet, P. van
5254 Caeter, C. R. Janssen, and H. F. De Brabander. 2007. Distribution and ecotoxicity of
5255 chlorotriazines in the Scheldt Estuary (B-Nl). Environmental Pollution (Barking, Essex :
5256 1987) 147:668–76.

5257 Norberg, J. 2004. Biodiversity and ecosystem functioning: a complex adaptive systems approach.
5258 Limnology and Oceanography 49:1269–1277.

5259 Novoveská, L., D. T. Franks, T. A. Wulfers, and W. J. Henley. 2016. Stabilizing continuous mixed
5260 cultures of microalgae. Algal Research 13:126–133.

5261 O'Connor, N. E., M. E. S. Bracken, T. P. Crowe, and I. Donohue. 2015. Nutrient enrichment alters the
5262 consequences of species loss. Journal of Ecology 103:862–870.

5263 O'Connor, N. E., and T. P. Crowe. 2005. Biodiversity loss and ecosystem functioning: distinguishing
5264 between number and identity of species. Ecology 86:1783–1796.

5265 Österblom, H., O. Olsson, T. Blenckner, and R. W. Furness. 2008. Junk-food in marine ecosystems.
5266 Oikos 117:967–977.

5267 Pacala, S., and A. P. Kinzig. 2002. Introduction to theory and the common ecosystem model. Pages
5268 169–174 *in* A. P. Kinzig, S. Pacala, and D. Tilman, editors. The functional consequences of
5269 biodiversity: empirical progress and theoretical extensions. Princeton University Press,
5270 Princeton, New Jersey.

5271 Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revelead in diverse

ecosystems. Trends in Ecology and Evolution 14:483–488.

Paffenhöfer, G. A., and M. Köster. 2005. Digestion of diatoms by planktonic copepods and doliolids. Marine Ecology Progress Series 297:303–310.

Pakeman, R. J. 2011. Multivariate identification of plant functional response and effect traits in an agricultural landscape. Ecology 92:1353–1365.

Parker, J. D., J. P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. Ecology Letters 13:553–563.

Parrish, C. C. 2009. Essential fatty acids in aquatic food webs. Pages 309–326 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York.

Pasari, J. R., T. Levi, E. S. Zavaleta, N. K. Tonthat, S. L. Milam, N. Chinnam, W. Margolin, M. a Schumacher, S. Sitch, M. A. White, and H. Hashimoto. 2013. Several scales of biodiversity affect ecosystem multifunctionality. Proceedings of the National Academy of Sciences 110:15163–15163.

Payne, M. F., R. J. Rippingale, and R. B. Longmore. 1998. Growth and survival of juvenile pipefish (*Stigmatopora argus*) fed live copepods with high and low HUFA content. Aquaculture 167:237–245.

Pennington, P., J. Daugomah, A. Colbert, M. Fulton, P. Key, B. Thompson, E. Strozier, and G. Scott. 2001. Analysis of pesticide runoff from mid-Texas estuaries and risk assessment implications for marine phytoplankton. Journal of Environmental Science and Health, Part B 36:1–14.

Pereira, H. M., P. W. Leadley, V. Proença, R. Alkemade, J. P. W. Scharlemann, J. F. Fernandez-Manjarrés, M. B. Araújo, P. Balvanera, R. Biggs, W. W. L. Cheung, L. Chini, H. D. Cooper, E. L. Gilman, S. Guénette, G. C. Hurtt, H. P. Huntington, G. M. Mace, T. Oberdorff, C. Revenga, P. Rodrigues, R. J. Scholes, U. R. Sumaila, and M. Walpole. 2010. Scenarios for global biodiversity in the 21st century. Science 330:1496–501.

Pérez, P., P. Estévez-Blanco, R. Beiras, and E. Fernández. 2006. Effect of copper on the photochemical efficiency, growth, and chlorophyll a biomass of natural phytoplankton assemblages. Environmental Toxicology and Chemistry 25:137–43.

Petchey, O. L., and K. J. Gaston. 2002a. Functional diversity (FD), species richness and community composition. Ecology Letters 5:402–411.

Petchey, O. L., and K. J. Gaston. 2002b. Extinction and the loss of functional diversity. Proceedings

of the Royal Society B-Biological Sciences 269:1721–7.

Petchey, O. L., T. Casey, L. Jiang, P. T. McPhearson, and J. Price. 2002c. Species richness, environmental fluctuations, and temporal change in total community biomass. Oikos 99:231–240.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters 9:741–758.

Petchey, O. L., K. J. Gaston, A. L. Downing, G. G. Mittelbach, L. Persson, C. F. Steiner, P. H. Warren, and G. Woodward. 2004. Species loss and the structure and functioning of multitrophic aquatic systems. Oikos 104:467–478.

Petchey, O. L., E. J. O'Gorman, and D. F. B. Flynn. 2009. A functional guide to functional diversity measures. Pages 49–60 *in* S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings, editors. Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective. Oxford University Press, Oxford.

Peterson, H. G., C. Boutin, P. A. Martin, K. E. Freemark, N. J. Ruecker, and M. J. Moody. 1994. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquatic Toxicology 28:275–292.

Piepho, M., M. T. Arts, and A. Wacker. 2012. Species-specific variation in fatty acid concentrations of four phytoplankton species: Does phosphorus supply influence the effect of light intensity or temperature? Journal of Phycology 48:64–73.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. Science 269:347–350.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: linear and nonlinear mixed effects models.

Pinho, G. L. L., M. S. Pedroso, S. C. Rodrigues, S. S. de Souza, and A. Bianchini. 2007. Physiological effects of copper in the euryhaline copepod *Acartia tonsa*: waterborne versus waterborne plus dietborne exposure. Aquatic Toxicology 84:62–70.

Pistocchi, R., F. Guerrini, V. Balboni, and L. Boni. 1997. Copper toxicity and carbohydrate production in the microalgae *Cylindrotheca fusiformis* and *Gymnodinium* sp.. European Journal of Phycology 32:125–132.

Plumley, G. F., and D. E. Davis. 1980. The effects of a photosynthesis inhibitor atrazine, on salt marsh edaphic algae, in culture, microecosystems, and in the field. Estuaries 3:271–277.

5334 Pohnert, G. 2005. Diatom/copepod interactions in plankton: the indirect chemical defense of
5335 unicellular algae. ChemBioChem 6:946–959.

5336 Pomati, F., N. J. B. Kraft, T. Posch, B. Eugster, J. Jokela, and B. W. Ibelings. 2013. Individual cell based
5337 traits obtained by scanning flow-cytometry show selection by biotic and abiotic
5338 environmental factors during a phytoplankton spring bloom. PLoS ONE 8:e71677.

5339 Pomati, F., B. Matthews, J. Jokela, A. Schildknecht, and B. W. Ibelings. 2012. Effects of re-
5340 oligotrophication and climate warming on plankton richness and community stability in a
5341 deep mesotrophic lake. Oikos 121:1317–1327.

5342 Pomati, F., and L. Nizzetto. 2013. Assessing triclosan-induced ecological and trans-generational
5343 effects in natural phytoplankton communities: a trait-based field method. Ecotoxicology
5344 22:779–794.

5345 Pondaven, P., M. Gallinari, S. Chollet, E. Bucciarelli, G. Sarthou, S. Schultes, and F. Jean. 2007.
5346 Grazing-induced changes in cell wall silicification in a marine diatom. Protist 158:21–28.

5347 Ponsatí, L., N. Corcoll, M. Petrović, Y. Picó, A. Ginebreda, E. Tornés, H. Guasch, D. Barceló, and S.
5348 Sabater. 2016. Multiple-stressor effects on river biofilms under different hydrological
5349 conditions. Freshwater Biology:2102–2115.

5350 Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, S. Mills, G. Daily, J. C. Castilla, J.
5351 Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. Bioscience 46:609–
5352 620.

5353 Purvis, A., and A. Hector. 2000. Getting the measure of well-being. Nature 405:212–219.

5354 Quigg, A., J. R. Reinfelder, and N. S. Fisher. 2006. Copper uptake kinetics in diverse marine
5355 phytoplankton. Limnology and Oceanography 51:893–899.

5356 Quijas, S., B. Schmid, and P. Balvanera. 2010. Plant diversity enhances provision of ecosystem
5357 services: A new synthesis. Basic and Applied Ecology 11:582–593.

5358 Rabaut, M., K. Guilini, G. Van Hoey, M. Vincx, and S. Degraer. 2007. A bio-engineered soft-bottom
5359 environment: The impact of *Lanice conchilega* on the benthic species-specific densities and
5360 community structure. Estuarine, Coastal and Shelf Science 75:525–536.

5361 Radchuk, V., F. De Laender, P. J. Van den Brink, and V. Grimm. 2016. Biodiversity and ecosystem
5362 functioning decoupled: invariant ecosystem functioning despite non-random reductions in
5363 consumer diversity. Oikos 125:424–433.

Raffaelli, D. 2004. How extinction patterns affect ecosystems. Ecology 306:5–6.

Raffaelli, D. 2006. Biodiversity and ecosystem functioning: issues of scale and trophic complexity. Marine Ecology Progress Series 311:285–294.

Raffaelli, D., M. van der Heijden, W. van der Putten, E. Kennedy, J. Koricheva, G. Lacroix, J. Mikola, L. Persson, O. Petchey, and D. A. Wardle. 2002. Multi-trophic processes and ecosystem function. Pages 147–154 *in* M. Loreau, S. Naeem, and P. Inchausti, editors. Biodiversity and ecosystem functioning. Oxford University Press, Oxford.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Real, M., I. Munoz, H. Guasch, E. Navarro, and S. Sabater. 2003. The effect of copper exposure on a simple aquatic food chain. Aquatic Toxicology 63:283–291.

Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. Science 336:589–592.

Reiss, J., J. R. Bridle, J. M. Montoya, and G. Woodward. 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in Ecology and Evolution 24:505–514.

Relyea, R., and J. Hoverman. 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters 9:1157–1171.

Rengel, Z. 1999. Heavy metals as essential nutrients. Pages 231–251 *in* M. N. V. Prasad and J. Hagemayer, editors. Heavy metal stress in plants. Springer, Berlin, Heidelberg.

Reusch, T. B. H., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences 102:2826–2831.

Rhee, J. S., I. T. Yu, B. M. Kim, C. B. Jeong, K. W. Lee, M. J. Kim, S. J. Lee, G. S. Park, and J. S. Lee. 2013. Copper induces apoptotic cell death through reactive oxygen species-triggered oxidative stress in the intertidal copepod *Tigriopus japonicus*. Aquatic Toxicology 132–133:182–189.

Ribalet, F., L. Intertaglia, P. Lebaron, and R. Casotti. 2008. Differential effect of three polyunsaturated aldehydes on marine bacterial isolates. Aquatic Toxicology 86:249–255.

Rijstenbil, J. W., J. W. M. Derksen, L. J. A. Gerringa, T. C. W. Poortvliet, A. Sandee, M. Vandenberg, J. Vandrie, and J. A. Wijnholds. 1994. Oxidative stress induced by copper: defense and damage

in the marine planktonic diatom *Ditylum brightwellii*, grown in continuous cultures with high and low zinc levels. Marine Biology 119:583–590.

Risgaard-Petersen, N., M. H. Nicolaisen, N. P. Revsbech, and B. A. Lomstein. 2004. Competition between ammonia-oxidizing bacteria and benthic microalgae. Applied and Environmental Microbiology 70:5528–5537.

Ritz, C., and J. C. Streibig. 2005. Bioassay analysis using R. Journal of Statistical Software 12:1–22.

Robinson, N. J. 1989. Algal metallothioneins: secondary metabolites and proteins. Journal of Applied Phycology 1:5–18.

Roger, F., A. Godhe, and L. Gamfeldt. 2012. Genetic diversity and ecosystem functioning in the face of multiple stressors. PLoS ONE 7.

Rohr, J. R., and P. W. Crumrine. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. Ecological Applications 15:1135–1147.

Rohr, J. R., J. L. Kerby, and A. Sih. 2006. Community ecology as a framework for predicting contaminant effects. Trends in Ecology and Evolution 21:606–613.

Rosen, D. A. S., and A. W. Trites. 2005. Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 175:265–273.

Rosi-Marshall, E. J., D. W. Kincaid, H. A. Bechtold, T. V. Royer, M. Rojas, and J. J. Kelly. 2013. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecological Applications 23:583–593.

Rubach, M. N., D. J. Baird, M. C. Boerwinkel, S. J. Maund, I. Roessink, and P. J. Van Den Brink. 2012. Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos. Ecotoxicology 21:2088–2101.

Sahan, E., K. Sabbe, V. Creach, G. Hernandez-Raquet, W. Vyverman, L. J. Stal, and G. Muyzer. 2007. Community structure and seasonal dynamics of diatom biofilms and associated grazers in intertidal mudflats. Aquatic Microbial Ecology 47:253–266.

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Skykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the Year 2100. Science 287:1770–1774.

Salleh, S., and A. McMinn. 2011. The effects of temperature on the photosynthetic parameters and recovery of two temperate benthic microalgae, *Amphora cf. coffeaeformis* and *Cocconeis cf. sublittoralis* (Bacillariophyceae). Journal of Phycology 47:1413–1424.

Sanpera-Calbet, I., I. Ylla, A. M. Romaní, S. Sabater, and I. Muñoz. 2016. Biochemical quality of basal resources in a forested stream: effects of nutrient enrichment. Aquatic Sciences:1–14.

Sargent, J. R., J. G. Bell, M. V. Bell, R. J. Henderson, and D. R. Tocher. 1995. Requirement criteria for essential fatty acids. Journal of Applied Ichthyology 11:183–198.

Sax, D. F., and S. D. Gaines. 2003. Species diversity: from global decreases to local increases. Trends in Ecology and Evolution 18:561–566.

Schabhüttl, S., P. Hingsamer, G. Weigelhofer, T. Hein, A. Weigert, and M. Striebel. 2013. Temperature and species richness effects in phytoplankton communities. Oecologia 171:527–536.

Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E.-D. Schulze, C. Roscher, A. Weigelt, E. Allan, H. Beßler, M. Bonkowski, N. Buchmann, F. Buscot, L. W. Clement, A. Ebeling, C. Engels, S. Halle, I. Kertscher, A.-M. Klein, R. Koller, S. König, E. Kowalski, V. Kummer, A. Kuu, M. Lange, D. Lauterbach, C. Middelhoff, V. D. Migunova, A. Milcu, R. Müller, S. Partsch, J. S. Petermann, C. Renker, T. Rottstock, A. Sabais, S. Scheu, J. Schumacher, V. M. Temperton, and T. Tscharntke. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468:553–556.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosysten function. Ecology 88:1386–1394.

Schläpfer, F., A. B. Pfisterer, and B. Schmid. 2005. Non-random species extinction and plant production: implications for ecosystem functioning. Journal of Applied Ecology 42:13–24.

Schläpfer, F., and B. Schmid. 1999. Ecosystem effects of biodiversity: a classification of hypotheses and exploration of empirical results. Ecological Applications 9:893–912.

Schmidtke, A., U. Gaedke, and G. Weithof. 2010. A mechanistic basis for underyielding in phytoplankton communities. Ecology 91:212–221.

Schneider, C. a, W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Schwarzman, M., and M. Wilson. 2009. New science for chemicals policy. Science 326:1065–1066.

5454 Siebenkäs, A., J. Schumacher, and C. Roscher. 2016. Effects in experimental grass-forb mixtures.
5455 PLoS ONE 11:1–21.

5456 Skene, K. R. 2004. Key differences in photosynthetic characteristics of nine species of intertidal
5457 macroalgae are related to their position on the shore. Canadian Journal of Botany 82:177–
5458 184.

5459 Smith, D. J., and G. J. C. Underwood. 2000. The production of extracellular carbohydrates by
5460 estaurine benthic diatoms: the effects of growth phase and light and dark treatment. Journal
5461 of Phycology 36:321–333.

5462 Smith, M. D., and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non-
5463 random species loss. Ecology Letters 6:509–517.

5464 Smith, R., R. Middlebrook, R. Turner, R. Huggins, S. Vardy, and M. Warne. 2012. Large-scale
5465 pesticide monitoring across Great Barrier Reef catchments - Paddock to Reef Integrated
5466 Monitoring, Modelling and Reporting Program. Marine Pollution Bulletin 65:117–127.

5467 Snelgrove, P. V. R. 1999. Getting to the bottom of marine biodiversity: Sedimentary habitats: Ocean
5468 bottoms are the most widespread habitat on Earth and support high biodiversity and key
5469 ecosystem services. BioScience 49:129–138.

5470 Snelgrove, P. V. R., S. F. Thrush, D. H. Wall, and A. Norkko. 2014. Real world biodiversity-ecosystem
5471 functioning: A seafloor perspective. Trends in Ecology and Evolution 29:398–405.

5472 Solan, M., B. J. Cardinale, A. L. Downing, K. a M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004.
5473 Extinction and ecosystem function in the marine benthos. Science 306:1177–80.

5474 Solan, M., J. A. Godbold, A. Symstad, D. F. Flynn, and D. E. Bunker. 2009. Biodiversity-ecosystem
5475 function research and biodiversity futures: early bird catches the worm or a day late and a
5476 dollar short. Pages 30–45 *in* S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings,
5477 editors. Biodiversity, ecosystem functioning, and human wellbeing: an ecological and
5478 economic perspective. Oxford University Press, Oxford.

5479 Soliveres, S., F. van der Plas, P. Manning, D. Prati, M. M. Gossner, S. C. Renner, F. Alt, H. Arndt, V.
5480 Baumgartner, J. Binkenstein, K. Birkhofer, S. Blaser, N. Blüthgen, S. Boch, S. Böhm, C. Börschig,
5481 F. Buscot, T. Diekötter, J. Heinze, N. Hölzel, K. Jung, V. H. Klaus, T. Kleinebecker, S. Klemmer,
5482 J. Krauss, M. Lange, E. K. Morris, J. Müller, Y. Oelmann, J. Overmann, E. Pašalić, M. C. Rillig, H.
5483 M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, J. Sikorski, S. A. Socher, E. F.
5484 Solly, I. Sonnemann, E. Sorkau, J. Steckel, I. Steffan-Dewenter, B. Stempfhuber, M. Tschapka,
5485 M. Türke, P. C. Venter, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, V.

5486 Wolters, T. Wubet, S. Wurst, M. Fischer, and E. Allan. 2016. Biodiversity at multiple trophic
5487 levels is needed for ecosystem multifunctionality. Nature 536:456–459.

5488 Solomon, K. R., J. a Carr, L. H. Du Preez, J. P. Giesy, R. J. Kendall, E. E. Smith, and G. J. Van Der Kraak.
5489 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Critical
5490 Reviews in Toxicology 38:721–72.

5491 Souffreau, C., P. Vanormelingen, E. Verleyen, K. Sabbe, and W. Vyverman. 2010. Tolerance of
5492 benthic diatoms from temperate aquatic and terrestrial habitats to experimental desiccation
5493 and temperature stress. Phycologia 49:309–324.

5494 Špaèková, I., and J. Lepš. 2001. Procedure for separating the selection effect from other effects in
5495 diversity-productivity relationship. Ecology Letters 4:585–594.

5496 Speybroeck, J., D. Bonte, W. Courtens, T. Gheskiere, P. Grootaert, J.-P. Maelfait, S. Provoost, K.
5497 Sabbe, E. W. M. Stienen, V. Van Lancker, W. Van Landuyt, M. Vincx, and S. Degraer. 2008. The
5498 Belgian sandy beach ecosystem: a review. Marine Ecology 29:171–185.

5499 Spijkerman, E., D. Barua, A. Gerloff-Elias, J. Kern, U. Gaedke, and S. A. Heckathorn. 2007. Stress
5500 responses and metal tolerance of *Chlamydomonas acidophila* in metal-enriched lake water
5501 and artificial medium. Extremophiles 11:551–562.

5502 Srivastava, D. S. 2002. The role of conservation in expanding biodiversity research. Oikos 98:351–
5503 360.

5504 Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P. Munguia, T.
5505 Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms useful model
5506 systems for ecology? Trends in Ecology and Evolution 19:379–384.

5507 Srivastava, D. S., and M. Vellend. 2005. Biodiversity - ecosystem functioning research: is it relevant
5508 to conservation? Annual Review of Ecology, Evolution, and Systematics 36:267–294.

5509 Staats, N., L. J. Stal, and L. R. Mur. 2000a. Exopolysaccharide production by the epipelic diatom
5510 *Cylindrotheca closterium*: effects of nutrient conditions. Journal of Experimental Marine
5511 Biology and Ecology 249:13–27.

5512 Staats, N., L. J. Stal, B. de Winder, and L. R. Mur. 2000b. Oxygenic photosynthesis as driving process
5513 in exopolysaccharide production of benthic diatoms. Marine Ecology Progress Series
5514 193:261–269.

5515 Stachova, T., P. Fibich, and J. Leps. 2013. Plant density affects measures of biodiversity effects.

Journal of Plant Ecology 6:1–11.

Stachowicz, J., J. Bruno, and J. Duffy. 2007. Understanding the effects of marine biodiversity on communities and ecosystems. Annual Review of Ecology, Evolution, and Systematics 38:739–766.

Stachowicz, J. J., R. J. Best, M. E. S. Bracken, and M. H. Graham. 2008a. Complementarity in marine biodiversity manipulations: reconciling divergent evidence from field and mesocosm experiments. Proceedings of the National Academy of Sciences of the United States of America 105:18842–7.

Stachowicz, J. J., M. Graham, M. E. S. Bracken, and A. I. Szoboszlai. 2008b. Diversity enhances cover and stability of seaweed assemblages: the role of heterogeneity and time. Ecology 89:3008–3019.

Stal, L. J. 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. Geomicrobiology Journal 20:463–478.

Stauber, J. L., and C. M. Davies. 2000. Use and limitations of microbial bioassays for assessing copper bioavailability in the aquatic environment. Environmental Reviews 8:255–301.

Stauber, J. L., and T. M. Florence. 1989. The effect of culture medium on metal toxicity to the marine diatom *Nitzschia closterium* and the freshwater green alga *Chlorella pyrenoidosa*. Water Research 23:907–911.

Stenersen, J. 2004. Chemical pesticides mode of action and toxicology. CRC Press, Boca Raton, Florida, USA.

Steudel, B., A. Hector, T. Friedl, C. Löfke, M. Lorenz, M. Wesche, and M. Kessler. 2012. Biodiversity effects on ecosystem functioning change along environmental stress gradients. Ecology Letters 15:1397–1405.

Stockenreiter, M., A. K. Graber, F. Haupt, and H. Stibor. 2012. The effect of species diversity on lipid production by micro-algal communities. Journal of Applied Phycology 24:45–54.

Stohs, S. J., and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biology & Medecine 18:321–336.

Suding, K. N., S. Lavorel, F. S. Chapin, J. H. C. Cornelissen, S. Díaz, E. Garnier, D. Goldberg, D. U. Hooper, S. T. Jackson, and M. L. Navas. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology 14:1125–1140.

5547 Symstad, A. J., and D. Tilman. 2001. Diversity loss, recruitment limitation, and ecosystem
5548 functioning: lessons learned from a removal experiment. Oikos 92:424–435.

5549 Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala, and M. T. Brett. 2013. Fatty acid
5550 composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in
5551 22 genera and in seven classes. Aquatic Microbial Ecology 71:165–178.

5552 Tang, M., and E. Kristensen. 2007. Impact of microphytobenthos and macroinfauna on temporal
5553 variation of benthic metabolism in shallow coastal sediments. Journal of Experimental
5554 Marine Biology and Ecology 349:99–112.

5555 Temperton, V. M., P. N. Mwangi, M. Scherer-Lorenzen, B. Schmid, and N. Buchmann. 2007. Positive
5556 interactions between nitrogen-fixing legumes and four different neighbouring species in a
5557 biodiversity experiment. Oecologia 151:190–205.

5558 Teoh, M. L., S. M. Phang, and W. L. Chu. 2013. Response of Antarctic, temperate, and tropical
5559 microalgae to temperature stress. Journal of Applied Phycology 25:285–297.

5560 Thebaut, E., and M. Loreau. 2005. Trophic interactions and the relationship between species. The
5561 American Naturalist 166:E95–E114.

5562 Thingstad, T. F., E. F. Skjoldal, and R. a Bohne. 1993. Phosphorus cycling and algal bacterial
5563 competition in Sandsfjord, western Norway. Marine Ecology Progress Series 99:239–259.

5564 Thompson, A. R. C., T. A. Norton, S. J. Hawkins, S. Ecology, and N. May. 2004. Physical Stress and
5565 Biological Control Regulate the Producer-Consumer Balance in Intertidal Biofilms. Ecology
5566 85:1372–1382.

5567 Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature 367:363–365.

5568 Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and ecosystem functioning. Annual
5569 Review of Ecology, Evolution, and Systematics 45:471–493.

5570 Tilman, D., C. L. Lehman, and K. T. Thomson. 1997. Plant diversity and ecosystem productivity:
5571 Theoretical considerations. Proceedings of the National Academy of Sciences 94:1857–1861.

5572 Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and
5573 productivity in a long-term grassland experiment. Science 294:843–845.

5574 Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity
5575 in grassland ecosystems. Nature 379:718–720.

5576 Tolhurst, T. J., K. S. Black, S. A. Shayler, S. Mather, I. Black, K. Baker, and D. M. Paterson. 1999.

Measuring the in situ erosion shear stress of intertidal sediments with the Cohesive Strength Meter (CSM). Estuarine, Coastal and Shelf Science 49:281–294.

Tolhurst, T. J., B. Jesus, V. Brotas, and D. M. Paterson. 2003. Diatom migration and sediment armouring — an example from the Tagus Estuary, Portugal. Pages 183–193 *in* M. B. Jones, A. Ingólfsson, E. Ólafsson, G. V Helgason, K. Gunnarsson, and J. Svavarsson, editors. Migrations and Dispersal of Marine Organisms SE - 20. CHAP, Springer Netherlands.

Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. Journal of the North American Benthological Society 26:509–522.

Trites, A. W., A. J. Miller, H. D. G. Maschner, M. A. Alexander, S. J. Bograd, J. A. Calder, A. Capotondi, K. O. Coyle, E. Di Lorenzo, B. P. Finney, E. J. Gregr, C. E. Grosch, S. R. Hare, G. L. Hunt, J. Jahncke, N. B. Kachel, H. J. Kim, C. Ladd, N. J. Mantua, C. Marzban, W. Maslowski, R. Mendelssohn, D. J. Neilson, S. R. Okkonen, J. E. Overland, K. L. Reedy-Maschner, T. C. Royer, F. B. Schwing, J. X. L. Wang, and A. J. Winship. 2007. Bottom-up forcing and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska: assessing the ocean climate hypothesis. Fisheries Oceanography 16:46–67.

Tuchman, N. C., M. a. Schollett, S. T. Rier, and P. Geddes. 2006. Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions. Hydrobiologia 561:167–177.

Turner, R. K., and M. Schaafsma. 2015. Coastal zones ecosystem services: from science to values and decision making. R. K. Turner and M. Schaafsma, editors. Springer.

Tylianakis, J. M., T. A. Rand, A. Kahmen, A. M. Klein, N. Buchmann, J. Perner, and T. Tscharntke. 2008. Resource heterogeneity moderates the biodiversity-function relationship in real world ecosystems. PLoS Biology 6:0947–0956.

Underwood, G. J. C., and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. Pages 93–153 *in* D. Nedwell and D. Raffaelli, editors. Advances in ecological research. Elsevier.

Underwood, G. J. C., and D. M. Paterson. 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. Advances in Botanical Research 40:183–240.

Underwood, G., J. Phillips, and K. Saunders. 1998. Distribution of estuarine benthic diatom species along salinity and nutrient gradients. European Journal of Phycology 33:173–183.

Van den Brink, P. J., A. C. Alexander, M. Desrosiers, W. Goedkoop, P. L. M. Goethals, M. Liess, and S. D. Dyer. 2011. Traits-based approaches in bioassessment and ecological risk assessment: Strengths, weaknesses, opportunities and threats. Integrated Environmental Assessment and Management 7:198–208.

Vanelslander, B., A. De Wever, N. Van Oostende, P. Kaewnuratchadasorn, P. Vanormelingen, F. Hendrickx, K. Sabbe, and W. Vyverman. 2009. Complementarity effects drive positive diversity effects on biomass production in experimental benthic diatom biofilms. Journal of Ecology 97:1075–1082.

Van Sprang, P., Vangheluwe, M., Van Hyfte, A., Heijerick, D., Vandenbroele, M., & Verdonck, F. 2007. European union risk assessment report: Copper, copper (II) sulphate pentahydrate, copper (I) oxide, copper (II) oxide, dicopper chloride trihydroxide. Voluntary risk assessment, Environmental effects, (Part 1).

Vansteenbrugge, L., K. Hostens, B. Vanhove, A. De Backer, L. De Clippele, and M. De Troch. 2016. Trophic ecology of *Mnemiopsis leidyi* in the southern North Sea: a biomarker approach. Marine Biology 163:1–17.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, Berlin.

Venail, P. A., R. C. Maclean, C. N. Meynard, and N. Mouquet. 2010. Dispersal scales up the biodiversity-productivity relationship in an experimental source-sink metacommunity. Proceedings of the Royal Society B: Biological Sciences 277:2339–2345.

Vinebrooke, R. D., K. L. Cottingham, J. Norberg, M. Scheffer, S. I. Dodson, S. C. Maberly, and U. Sommer. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Pages 451–457Oikos.

Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos 116:882–892.

Vitousek, P., and D. Hooper. 1993. Biodiversity and ecosystem function. Pages 3–14 *in* E. D. Schulze and H. Mooney, editors. Springer, Berlin.

Vitousek, P. M., H. a Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth' s ecosystems. Science 277:494–499.

von Elert, E. 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnology and Oceanography 47:1764–1773.

5639 Wagg, C., S. F. Bender, F. Widmer, and M. G. A. van der Heijden. 2014. Soil biodiversity and soil
5640 community composition determine ecosystem multifunctionality. Proceedings of the
5641 National Academy of Sciences of the United States of America 111:5266–70.

5642 Wallace, K. J. 2007. Classification of ecosystem services: Problems and solutions. Biological
5643 Conservation 139:235–246.

5644 Wang, Y., J. Lu, J.-C. Mollet, M. R. Cretz, and K. D. Hoagland. 1997. Extracellular matrix assembly in
5645 diatoms (Bacillariophyceae). Plant Physiology 113:1059–1069.

5646 Wanless, S., M. P. Harris, P. Redman, and J. R. Speakman. 2005. Low energy values of fish as a
5647 probable cause of a major seabird breeding failure in the North Sea. Marine Ecology Progress
5648 Series 294:1–8.

5649 Wainman, B. C., R. E. Smith, H. Rai, and J. A. Furgal. 1999. Irradiance and lipid production in natural
5650 algal populations.Pages 45–70 *in* M. T. Arts and B. C. Wainmann, editors. Lipids in freshwater
5651 ecosystems. Springer, New York.

5652 Waldbusser, G. G., R. L. Marinelli, R. B. Whitlatch, and P. T. Visscher. 2004. The effects of infaunal
5653 biodiversity on biogeochemistry of coastal marine sediments. Limnology and Oceanography
5654 49:1482–1492.

5655 Wang, J., C. B. Zhang, T. Chen, and W. H. Li. 2013. From selection to complementarity: the shift
5656 along the abiotic stress gradient in a controlled biodiversity experiment. Oecologia 171:227–
5657 235.

5658 Wang, Y., and M. Frei. 2011. Stressed food - The impact of abiotic environmental stresses on crop
5659 quality. Agriculture, Ecosystems and Environment 141:271–286.

5660 Wardle, D. A. 1999. Is 'sampling effect' a problem for experiments investigating biodiversity-
5661 ecosystem function relationships? Oikos 87:403–407.

5662 Wardle, D. A. 2016. Do experiments exploring plant diversity-ecosystem functioning relationships
5663 inform how biodiversity loss impacts natural ecosystems? Journal of Vegetation Science
5664 27:646–653.

5665 Weisshaar, H., G. Retzlaff, and P. B. Boger. 1988. Chloroacetamide inhibition of fatty acid synthesis.
5666 Pesticide Biochemistry and Physiology 32:212–216.

5667 Welsh, P. G., J. L. Parrott, D. G. Dixon, P. V. Hodson, D. J. Spry, and G. Mierle. 1996. Estimating acute
5668 copper toxicity to larval fathead minnow (*Pimphales promelas*) in soft water from
5669 measurements of dissolved organic carbon, calcium, and pH. Canadian Journal of Fisheries

and Aquatic Sciences 53:1263–1271.

Wendt-Rasch, L., P. J. Van Den Brink, S. J. H. Crum, and P. Woin. 2004. The effects of a pesticide mixture on aquatic ecosystems differing in trophic status: responses of the macrophyte *Myriophyllum spicatum* and the periphytic algal community. Ecotoxicology and Environmental Safety 57:383–398.

Werbrouck, E., D. Van Gansbeke, A. Vanreusel, C. Mensens, and M. De Troch. 2016. Temperature-induced changes in fatty acid dynamics of the intertidal grazer *Platychelipus littoralis* (Crustacea, Copepoda, Harpacticoida): Insights from a short-term feeding experiment. Journal of Thermal Biology 57:44–53.

Wickham, J. D. 1997. A Conceptual Framework for Selecting and Analyzing Stressor Data to Study Species Richness at Large Spatial Scales. Environmental Management 21:247–257.

Wilcove, D. S., and L. L. Master. 2005. How many endangered species are there in the United States? Frontiers in Ecology and the Environment 3:414.

Wilsey, B. J., and H. W. Polley. 2004. Realistically low species evenness does not alter grassland species-richness-productivity relationships. Ecology 85:2693–2700.

Wittebolle, L., M. Marzorati, L. Clement, A. Balloi, D. Daffonchio, P. De Vos, K. Heylen, W. Verstraete, and N. Boon. 2009. Initial community evenness favours functionality under selective stress. Nature 458:623–6.

Woin, P. 1998. Short- and Long-Term Effects of the Pyrethroid Insecticide Fenvalerate on an Invertebrate Pond Community. Ecotoxicology and environmental safety 41:137–146.

Wolfstein, K., and L. J. Stal. 2002. Production of extracellular polymeric substances (EPS) by benthic diatoms: Effect of irradiance and temperature. Marine Ecology Progress Series 236:13–22.

Wood, R. J., S. M. Mitrovic, and B. J. Kefford. 2014. Determining the relative sensitivity of benthic diatoms to atrazine using rapid toxicity testing: A novel method. The Science of the Total Environment 485–486C:421–427.

Worm et al., B. 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–790.

Wu, J., S. Chang, and K. Chen. 1995. Enhancement of intracellular proline level in cells of *Anacystis nidulans* (cyanobacteria) exposed to deleterious concentrations of copper. Journal of Phycology 379:376–379.

Wu, Z. X., Gan, N. Q, Huang, Q., and L. R. Song. 2007. Response of Microcystis to copper stress - do phenotypes of *Microcystis* make a difference in stress tolerance? Environmental Pollution 147:324–330.

Wyckmans, M., V. a. Chepurnov, A. Vanreusel, and M. De Troch. 2007. Effects of food diversity on diatom selection by harpacticoid copepods. Journal of Experimental Marine Biology and Ecology 345:119–128.

Yarmin, F. 1995. Biodiversity, ethics and international law. International Affairs 721:529–546.

Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. Science 306:1175–7.

Zhang, A. Q., K. M. Y. Leung, K. W. H. Kwok, V. W. W. Bao, and M. H. W. Lam. 2008. Toxicities of antifouling biocide Irgarol 1051 and its major degraded product to marine primary producers. Marine Pollution Bulletin 57:575–586.

Zhang, Y., H. Y. H. Chen, and P. B. Reich. 2012. Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. Journal of Ecology 100:742–749.

Zwart, J. A., C. T. Solomon, and S. E. Jones. 2015. Phytoplankton traits predict ecosystem function in a global set of lakes. Ecology 96:2257–2264.